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Applicant:	Eugene R. Cooper et al.
Title:	NANOPARTICULATE MELOXICAM FORMULATIONS
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DECLARATION UNDER 37 C.F.R. §1.132

The undersigned, Gary G. Liversidge, hereby declares as follows:

I. Background of Gary G. Liversidge

1. I received my Ph.D. in 1981 from the University of Nottingham, England, in Pharmaceutical Chemistry. I have been working in the field of nanoparticulate drug technology since 1987, when I joined Eastman Pharmaceuticals.

2. Through a series of business transactions, Eastman Pharmaceuticals became Sterling Winthrop Pharmaceuticals Research Division, and then NanoSystems. This business then became known as the Elan Drug Technologies (EDT) business division of Elan Corp. PLC. EDT merged with Alkermes, Inc. on September 8, 2011 to form Alkermes, plc. Through a series of business transactions the current owner of the present application is Alkermes Pharma Ireland Limited.

3. Currently I am Chief Technology Officer and Vice President, R&D, at Alkermes plc, based at 852 Winter Street, Waltham, MA 02451.

II. Not all active agents can be formulated into stable nanoparticulate active agent compositions

A. It is unpredictable whether a functional equivalent can be successfully made into a nanoparticulate active agent composition.

4. It is unpredictable whether a functional equivalent of a compound can be successfully made into a nanoparticulate active agent composition. This fact is demonstrated by the data below, detailing the successful preparation of a stable nanoparticulate cilostazol composition, in contrast to the unsuccessful attempts to make a stable nanoparticulate clopidogrel bisulphate composition. Clopidogrel is an inhibitor of platelet aggregation, and is a functional equivalent of cilostazol in inhibiting platelet aggregation.

(1) Stable nanoparticulate cilostazol composition was successfully obtained.

5. Cilostazol is a drug used to inhibit platelet aggregation. *See* abstract by Kimura et al., *Arzneimittelforschung*, *35*(7A): 1144-1149 (1985) (Exhibit 1). Cilostazol is a poorly water soluble drug having an aqueous solubility of 3 μg/mL at 25°C or 6 μg/mL at 37°C.

6. As demonstrated by Jinno et al., *J. Controlled Release*, *111*: 56-64 (2006) (Exhibit 2), a stable nanoparticulate cilostazol composition was successfully obtained by Elan's (now Alkermes') NanoCrystal® technology. *See* page 57, right column, section 2.3 for "Preparation of cilostazol suspensions." The obtained nanoparticulate cilostazol composition comprised hydroxypropyl cellulose and docusate sodium as the surface stabilizers. *Id.*, section 2.2 for "Particle size reduction of cilostazol." The nanoparticulate cilostazol composition had a median particle size of 0.22 μm (or 220 nm). *See* page 59, Figure 1, and section 3.1 for "Size distribution of milled cilostazol."

7. Exhibit 2 further demonstrates superior solubility, dissolution, bioavailability, and elimination of food effect for the nanoparticulate cilostazol composition in comparison to the

hammer-milled and jet-milled cilostazol compositions having a median particle size of 13 μm and 2.4 μm, respectively. *See* pages 59-63.

8. Accordingly, a nanoparticulate cilostazol composition was successfully obtained using NanoCrystal® technology, as demonstrated by the published scientific literature.

(2) Stable nanoparticulate clopidogrel bisulphate composition could not be obtained.

9. Clopidogrel is an inhibitor of platelet aggregation, and is a functional equivalent of cilostazol in inhibiting platelet aggregation. Clopidogrel bisulphate is insoluble in water at neutral pH but freely soluble in water at a pH of 1.0. Clopidogrel, as a methyl ester, is hydrolysed *in vivo* by esterases to an inactive carboxylic acid derivative, which represents more than 85% of the circulating drug related compounds in the plasma. Thus, only a small unknown portion of clopidogrel is available for metabolism to the active metabolite after oral administration. Therefore, it is highly desirable to obtain a stable nanoparticulate clopidogrel bisulphate composition to improve bioavailability of this drug.

10. The challenge in obtaining a stable nanoparticulate clopidogrel bisulphate composition is that during the milling process to reduce the particle size of clopidogrel, the pH of the milling mixture decreases while the dissolution of clopidogrel bisulphate increases. Accordingly, clopidogrel bisulphate undergoes auto-catalysis during the milling process until it is completely dissolved at pH 1-2. Solubilizing clopidogrel bisulphate does not solve the problem of poor bioavailability as *in vivo* the drug is hydrolysed by esterases to an inactive carboxylic acid derivative, as noted above.

11. Using the same NanoCrystal® technology, different approaches were attempted to stabilize particulate clopidogrel bisulphate during the milling process, such as milling in buffered systems and milling using the common ion effect, although none of these approaches resulted in a stable nanoparticulate clopidogrel bisulphate composition.

12. In a first set of experiments, milling of clopidogrel bisulphate was conducted in different buffered systems having a pH from 6.0 – 12.0 to prevent dissolution of clopidogrel bisulphate during milling. The results are detailed in the table below.

Table 1				
Clopidogrel Bisulphate (% w/w)	**Surface Stabilizer (% w/w)**	**Buffered System (pH)**	**pH prior to milling**	**pH post milling**
5%	HPC-SL (2%)	93% w/w citric acid/sodium phosphate dibasic solution (pH 7.0)	not determined	2.21
5%	Plasdone S-630 (2%)	93% w/w sodium phosphate monobasic/sodium phosphate dibasic solution (pH 8.0)	7.0	1.82
5%	Plasdone K29/K32 (2%)	93% w/w hydrochloric acid-Tris (hydroxymethyl) amino methane solution (pH 8.9)	2.0	Not milled due to dissolution of active agent prior to milling
5%	HPC-SL (2%)	93% w/w citric acid/sodium phosphate dibasic solution (pH 7.0)	6.0	2.17
5%	HPC-SL (2%)	93% w/w buffered solution (pH 12.0)	3.0	Not milled due to dissolution of active agent prior to milling
5%	Pharmacoat 603 (2%)	93% w/w simulated intestinal fluid (pH 12.0)	Not determined	2.0

13. Due to the dissolution of clopidogrel bisulphate in the buffered systems either prior to or post milling, despite the variable conditions attempted, it was found that it was ***impossible*** to obtain a stable nanoparticulate clopidogrel bisulphate compositions.

14. In a second set of experiments, milling of clopidogrel bisulphate was conducted under the condition of saturating the system with common ion, bisulfate ion, in a saturated

sodium bisulphate solution to control the equilibrium solubility of the clopidogrel during milling. The results are detailed in the table below.

Table 2

Clopidogrel Bisulphate (% w/w)	Surface Stabilizer (% w/w)	Observations
5%	None	Microscopy showed the presence of drug particles in small quantities. The majority of material observed was in a flocculated state.
5%	Pharmacoat 603 (HPMC) (1%)	After subsequent addition of stabilizer, flocculation appeared to be reduced but still present. An increased proportion of the drug appeared in the harvested aliquot suggesting increased milling of the drug. Brownian motion was not apparent.
5%	HPC-SL (2%)	Some milled drug particles were apparent although in very small quantities. Particles did not exhibit Brownian motion. Flocculation was readily apparent throughout the aliquot of sample observed under microscope.
5%	Tween 80 (2%)	Unmilled drug particles apparent. Aliquot harvested for microscopy was extremely dilute suggesting that very little drug was milled.
5%	Pharmacoat 603 (HPMC) (2%)	Nanoparticles were observed in very small quantities. Particle did exhibit a small degree of Brownian motion.
5%	Pharmacoat 603 (HPMC) (2%), DOSS (0.05%)	Very small quantities of milled drug particles were observed. Although particles were somewhat milled, they did not appear to be in the nanoparticulate size range. No Brownian motion was observed
5%	Plasdone S-630 (2%)	Harvesting was not possible as the drug did not appear to mill. No microscopy was therefore performed. A large proportion of the slurry was observed on upper mill plate and agitator possibly leading to a void in the mill chamber reducing the milling efficiency.
5%	Tyloxapol (1%)	Microscopy showed the presence of a very small concentration of milled particulates which appeared to exhibit Brownian motion most likely due to the diluted nature of the slurry.

15. Despite the variable conditions attempted, it was found that it was ***impossible*** to obtain a stable nanoparticulate clopidogrel composition by controlling the equilibrium solubility of clopidogrel bisulphate via saturating the system with the common ion.

16. Accordingly, a stable nanoparticulate clopidogrel bisulphate composition could not be obtained using the NanoCrystal® technology under the various conditions tested.

B. Stable nanoparticulate orlistat compositions could not be obtained.

17. Orlistat is a drug for treating obesity by preventing the absorption of fats from diet, thereby reducing caloric intake. However, orlistat is associated with significant gastrointestinal side effects, including steatorrhea, fecal incontinence, and frequent or urgent bowel movements. Therefore, it is desirable to obtain a nanoparticulate orlistat composition to alleviate the side effects.

18. The challenge in obtaining a stable nanoparticulate orlistat composition is that despite the attempts to mill approximately 30 different orlistat formulations, it was very challenging to obtain a nanoparticulate orlistat composition having the desired particle size due to a number of problems, such as difficulty encountered to "wet in" orlistat for milling, a significant quantity of unmilled orlistat remaining in the milling chamber, and difficulty encountered to harvest the milled orlistat.

19. Even when a nanoparticulate orlistat composition having the desired particle size was initially obtained, the composition was unstable under common storage conditions for a period of 14 days, as detailed in the table below.

Table 3

Formulation	Storage Time (days)	Storage Condition	Mean (nm)	D50 (nm)	D90 (nm)	D95 (nm)
Orlistat, 5%w/w Pharmacoat 603, 2%w/w (Hydroxypropyl methylcellulose) Deionised Water, 93%w/w	0	N/A	436	339	658	1009
	0	N/A	390	334	599	795
	14	**5°C**	**50732**	**23999**	**139683**	**178902**
	14	**5°C**	**2045**	**362**	**7376**	**13593**
Orlistat, 5%w/w Pharmacoat 603, 1.25%w/w (Hydroxypropyl methylcellulose) Lauryl Sulfate, 0.05%w/w (Sodium Lauryl Sulfate) Deionised Water, 93.7%w/w	0	N/A	196	185	258	295
	0	N/A	195	185	257	294
	14	**5°C**	**28076**	**196**	**100516**	**136455**
	14	**5°C**	**2439**	**208**	**8771**	**18960**
	14	**25°C/60% RH**	**16072**	**170**	**72678**	**104171**
	14	25°C/60% RH	1214	212	717	7838
Orlistat, 5%w/w Pluronic F108, 1.5%w/w (Poloxamer 338) Deionised Water, 93.5%w/w	0	N/A	649	296	1907	2925
	0	N/A	580	292	1487	2628
	14	**5°C**	**38717**	**15805**	**106358**	**133797**
	14	5°C	314	164	286	693
Orlistat, 5%w/w Pharmacoat 603, 1.25%w/w (Hydroxypropyl methylcellulose) Lauryl Sulfate, 0.05%w/w (Sodium Lauryl Sulfate) Deionised Water, 93.7%w/w	0	N/A	400	289	537	1017
	0	N/A	340	286	485	673
	14	**5°C**	**64545**	**4924**	**209398**	**266403**
	14	**5°C**	**3405**	**311**	**12049**	**24135**
	14	**25°C/60% RH**	**13556**	**189**	**58786**	**88122**
	14	25°C/60% RH	814	211	984	3870
Orlistat, 5%w/w Pluronic F108, 1.00%w/w (Poloxamer 338) Tween 80, 1.00%w/w (Polyoxyethylene Sorbitan Fatty Acid Esters) Deionised Water, 93%w/w	0	N/A	176	164	225	261
	0	N/A	168	161	219	246
	14	5°C	1790	152	486	8500
	14	5°C	219	194	272	345
	14	**25°C/60% RH**	**22241**	**371**	**90884**	**120263**
	14	25°C/60% RH	651	284	485	2949
Orlistat, 5%w/w HPC-SL, 1.25%w/w (Hydroxypropyl Cellulose-Super Low Viscosity) Docusate Sodium, 0.05%w/w (Docusate Sodium) Deionised Water, 93.7%w/w	0	N/A	232	174	318	537
	0	N/A	263	217	371	532
	14	**5°C**	**40976**	**265**	**130336**	**174898**
	14	**5°C**	**3110**	**216**	**10063**	**22230**
	14	**25°C/60% RH**	**22255**	**170**	**84494**	**118656**
	14	25°C/60%	506	192	302	2102

Table 3						
Formulation	**Storage Time (days)**	**Storage Condition**	**Mean (nm)**	**D50 (nm)**	**D90 (nm)**	**D95 (nm)**
		RH				
Orlistat, 5%w/w HPC-SL, 2%w/w (Hydroxypropyl Cellulose-Super Low Viscosity) Deionised Water, 93%w/w	0	N/A	419	330	639	971
	0	N/A	438	331	671	1100
	14	**5°C**	**76195**	**64261**	**175942**	**221539**
	14	**5°C**	**5418**	**399**	**19307**	**26735**
	14	**25°C/60% RH**	**29931**	**19086**	**78580**	**96406**
	14	25°C/60% RH	1211	310	3568	7161
Orlistat, 5%w/w Lutrol F127, 1.5%w/w (Poloxamer 407) Deionised Water, 93.5%w/w	0	N/A	347	205	436	1330
	0	N/A	265	192	314	617
	14	**5°C**	**6161**	**167**	**24954**	**46361**
	14	5°C	219	192	271	344
Orlistat, 5%w/w HPC-SL, 2%w/w (Hydroxypropyl Cellulose-Super Low Viscosity) Deionised Water, 93%w/w	0	N/A	232	174	318	537
	0	N/A	263	217	371	532
	14	**5°C**	**40976**	**265**	**130336**	**174898**
	14	**5°C**	**3110**	**216**	**10063**	**22230**
	14	**25°C/60% RH**	**22255**	**170**	**84494**	**118656**
	14	25°C/60% RH	506	192	302	2102

20. Accordingly, a stable nanoparticulate orlistat composition could not be obtained under the various conditions tested.

C. Banavath

21. The technologies employed to obtain nanoparticulate active agent compositions, such as precipitation, microemulsion, high pressure homogenization, and milling, are all associated with disadvantages. *See* Banavath et al., "Nanosuspension: An Attempt To Enhance Bioavailability Of Poorly Soluble Drugs," *Int'l J. Pharm. Sci. and Res.*, *1*(9): 1-11 (2011) (Exhibit 3), at page 4, Table 2.

22. More specifically, precipitation may cause the growth of drug crystals and requires that the drug be soluble in at least one solvent. Microemulsion requires the use of a high

amount of surfactant and stabilizer, which increases production cost. At times, microemulsion even involves the use of hazardous solvents in production. Homogenization requires that the drug be pre-processed into a micronized state, and possible contamination may occur from metal ions from the wall of the homogenizer. Milling is a time-consuming process which is hard to scale up and which may have contamination from the milling media. Also prolonged milling may induce instability of the drug, resulting in the drug transforming into an amorphous state. Therefore, not all active agents can be successfully made into nanoparticulate active agent formulations in view of the technologies available to date.

D. Wu

23. Wu et al. ("Physical and chemical stability of drug nanoparticles," *Advanced Drug Delivery Reviews*, electronically published in February, 2011, Exhibit 4) report that it remains challenging to obtain nanoparticulate active agent compositions that are physically and chemically stable because the stability is affected by many factors. *See* lines 84-105 and 855-861.

24. More specifically, Wu et al. teach that obtaining a stable nanoparticulate active agent composition is hindered by the difficulty of selecting a suitable surface stabilizer for the active agent. Moreover, according to Wu et al. the main challenges in designing nanoparticulate drug formulations are: (i) the lack of a fundamental understanding of the interaction between the surface stabilizer and the active agent nanoparticles (*see* lines 268-273); (ii) the process of selecting a surface stabilizer having an appropriate anchoring tail to the particular active agent is burdensome (*see* lines 268-273); (iii) the lack of predictability due to the lack of any correlation between the physiochemical properties of the active agent and the success rate of obtaining a stable nanoparticulate active agent composition (*see* lines 399-402); and (iv) the lack of an efficient and high throughput screening technique to identify a suitable surface stabilizer (*see* lines 812-816).

III. Nanoparticulate formulations do not always improve bioavailability of the active agent in comparison to other non-nanoparticulate formulations

25. U.S. Patent No. 7,217,431 (Exhibit 5) demonstrates that a nanoparticulate formulation of a drug substance according to Elan's (now Alkermes') nanotechnology does not improve *in vivo* bioavailability of the drug in comparison to other non-nanoparticulate formulations of the same drug substance. *See* Example 4, which is summarized in the following paragraphs. This data therefore demonstrates that a researcher cannot predict whether an active agent will exhibit an improved pharmacokinetic profile by reformulating the active agent into a nanoparticulate formulation.

26. Five different formulations of a drug substance, including a nanosuspension of the drug supplied by Elan (the prior assignee of the present application), were orally administered to dogs at similar doses and then tested for *in vivo* bioavailability. *See* column 24, line 61 through column 25, line 37. The nanosuspension of the drug comprised nanoparticulate drug (e.g., drug particles having an effective average particle size of less than 1 micron), HPC-SL as a surface stabilizer adsorbed to the surface of the drug particles, and water. The particulate material of formulation B or C had a geometric weight mean diameter between 75 μm and 2000 μm. *See* column 38, claim 1.

Table 4		
Formulations	**Contents**	**Dose**
A	Nanosuspension stabilized by hydroxyl propyl cellulose (HPC-SL) (supplied by Elan)	36.3 mg
B	Tablets containing a particulate material of the drug	37.5 mg
C	Tablets containing a particulate material of the drug	42.4 mg
D	Capsules containing a microemulsion of the drug	36.5 mg
E	Capsules containing a microemulsion of the drug	37.2 mg

27. Despite the minor variation in the dose of each formulation administered to dogs, the non-nanoparticulate tablet and capsule formulations achieved higher *in vivo* bioavailability, as represented by higher C_{max} and AUC, in comparison to the nanosuspension of the same drug substance. *See* the table spanning columns 27 and 28, the results of which are summarized in the table below.

Table 5				
Formulations	**C_{max}**		**AUC**	
	C_{max} (ng/ml)	**% relative to Formulation A**	**AUC_{0-inf} (ng/ml)**	**% relative to Formulation A**
A	19 ± 8	--	206 ± 108	--
B	52 ± 15	274%	489 ± 187	237%
C	29 ± 17	153%	290 ± 184	141%
D	35 ± 13	184%	318 ± 144	154%
E	42 ± 6	221%	318 ± 65	154%

28. Accordingly, both non-nanoparticulate capsule and tablet formulations containing drug particles of much larger particle size exhibited ***greater*** bioavailability as compared to a nanoparticulate formulation, as demonstrated by Exhibit 5.

IV. Unexpected results achieved by the claimed invention

29. To date, all meloxicam formulations approved by the U.S. Food and Drug Administration (FDA) are oral formulations, as demonstrated by Exhibit 6 (printed from the online Orange Book, meloxicam).

30. Potentially serious gastrointestinal irritation is an adverse effect associated with oral dosage forms of meloxicam. In fact, FDA requires that the package insert of the prescription drug meloxicam to carry a black box warning of the serious side effects, including gastrointestinal risk. *See* Meloxicam FDA Warning (Exhibit 7), page 1.

31. Thus, it is highly desirable to develop a meloxicam formulation suitable for intravenous injection, particularly for patient populations that have difficulty in ingesting an oral dosage form, such as pediatric patient populations, and for patient populations that experience serious side effect of gastrointestinal irritation.

32. An oral dosage formulation of meloxicam has a slow onset relative to an intravenous formulation of the same active agent. To demonstrate this fact, a commercial oral non-nanoparticulate meloxicam dosage form was compared to an intravenous nanoparticulate meloxicam dosage form.

33. The commercial oral microparticulate meloxicam dosage form used in the experiments described below is sold under the trade name MOBIC® by Boehringer Ingelheim (*see* Exhibit 6).

34. The nanoparticulate meloxicam composition comprised 2.5% (w/w) meloxicam, as well as 0.75% (w/w) polyvinylpyrrolidone and 0.25% (w/w) sodium deoxycholate as surface stabilizers and had an effective average particle size of 104 nm and a D_{90} particle size of 129 nm.

35. MOBIC® 15 mg tablets were orally administered, or the nanoparticulate meloxicam composition was intravenously administered, to 3 cohorts (6 subjects in each cohort) at doses of 15 mg, 30 mg and 60 mg, respectively. The pharmacokinetic parameters were monitored and detailed in the table below.

Table 6: Pharmacokinetic Profiles of MOBIC® and Nanoparticulate Meloxicam Formulation

Cohort/Dose	Pharmacokinetics	MOBIC® (oral tablet)	Nanoparticulate meloxicam (IV)	Relative Bioavailability (IV/oral tablet)
#1 (15 mg meloxicam)	AUC_{inf} (ng*hr/mL)	42949	46095	107%
	C_{max} (ng/mL)	1222	n/a	n/a
	T_{max} (hour)	6.57	n/a	n/a
#2 (30 mg meloxicam)	AUC_{inf} (ng*hr/mL)	104400	107509	103%
	C_{max} (ng/mL)	2611	n/a	n/a
	T_{max} (hour)	6.86	n/a	n/a
#3 (60 mg meloxicam)	AUC_{inf} (ng*hr/mL)	163855	171229	105%
	C_{max} (ng/mL)	4810	n/a	n/a
	T_{max} (hour)	5.14	n/a	n/a

36. As shown in Table 6, the intravenously administered nanoparticulate meloxicam formulation consistently achieved the same or slightly improved AUC as the MOBIC® 15 mg tablet orally administered, at all doses tested.

37. The C_{max} and T_{max} for the nanoparticulate meloxicam formulation were not monitored because an intravenously administered formulation is expected to achieve a C_{max} (peak plasma concentration of drug following administration) immediately after completion of administration. Therefore, the T_{max} (the time after administration of a drug when the C_{max} is

reached) of an intravenously administered formulation is expected to be immediately after completion of administration.

38. In contrast, the orally administered MOBIC® tablet has a ***significantly slower onset***, reflected by a T_{max} range from 5.14 hours to 6.86 hours. This is significant because meloxicam is prescribed to relieve pain, tenderness, swelling, and stiffness caused by osteoarthritis or juvenile rheumatoid arthritis in children 2 years of age and older. *See* the PubMed Health publication regarding meloxicam (Exhibit 8). Particularly for very young children, the existing oral meloxicam formulations cause difficulty in administration and the slow onset of oral meloxicam formulations fails to benefit this particular patient population.

CONCLUSION

39. The data described herein demonstrate unpredictability in the art, such that there is no *a priori* expectation that any given active agent could be made into a nanoparticulate active agent composition, even when a functional equivalent of the active agent has successfully been made into a nanoparticulate active agent composition. The data also shows that it is incorrect to assume that improved bioavailability will result merely from making the nanoparticulate form of an active agent. Finally, the data demonstrated the unexpected results achieved by the claimed invention, that is the claimed intravenous dosage form of nanoparticulate meloxicam composition was able to achieve significantly improved onset (achieving the same plasma concentration within much shorter time period) in comparison to the commercially available oral formulations.

40. I declare that the statements made herein of my knowledge are true and all statements on information and belief are believed to be true; and further these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing therein.

Gary G. Liversidge

2/15/12
Date

Enclosures:
Publication by Kimura et al. (Exhibit 1);
Publication by Jinno et al. (Exhibit 2);
Publication by Banavath et al (Exhibit 3);
Publication by Wu et al. (Exhibit 4);
U.S. Patent No. 7,217,431 (Exhibit 5)
Orange Book, meloxicam (Exhibit 6)
Meloxicam FDA Warning (Exhibit 7)
PubMed Health publication regarding meloxicam (Exhibit 8)

EXHIBIT 1

PubMed "Arzneimittel-Forschung"[Jour] AND 35[volume] AND 1144 Search

Display Settings: Abstract

Arzneimittelforschung. **1985**;**35**(7A):**1144**-9.

Effect of cilostazol on platelet aggregation and experimental thrombosis.

Kimura Y, Tani T, Kanbe T, Watanabe K.

Abstract

A new antithrombotic drug, cilostazol (6-[4-(1-cyclohexyl-1 H-tetrazol-5-yl)butoxy]-3,4-dihydro-2(1H)-quinolinone, OPC-13013) was studied for its inhibitory effect on platelet aggregation in vitro in various experimental animals and man and in dogs ex vivo, for its effect to disperse platelet aggregates in vitro in rabbits and man and for its antithrombotic effect in vivo using its effect to prevent death due to the formation of pulmonary thrombi in mice. Cilostazol produced a potent inhibition of platelet aggregation both in vitro and ex vivo and a dispersion of platelet aggregates in vitro. The mode of action of cilostazol was different from that of acetylsalicylic acid (ASA) in that cilostazol inhibits not only secondary platelet aggregation but also primary platelet aggregation induced by aggregating agents such as adenosine diphosphate (ADP). The drug potently prevented death due to pulmonary thrombosis by platelet aggregates in mice in vivo. Unlike ASA which prevented only death due to collagen-induced platelet aggregation, cilostazol prevented both collagen- and ADP-induced platelet aggregation. These results suggest that cilostazol is a promising antithrombotic drug.

PMID:4074426[PubMed - indexed for MEDLINE]

Publication Types, MeSH Terms, Substances

LinkOut - more resources

EXHIBIT 2

Available online at www.sciencedirect.com
SCIENCE DIRECT
Journal of Controlled Release 111 (2006) 56–64
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www.elsevier.com/locate/jconrel

Effect of particle size reduction on dissolution and oral absorption of a poorly water-soluble drug, cilostazol, in beagle dogs

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Abstract

The purpose of the present study was to investigate the effects of particle size on the dissolution and oral absorption of cilostazol. Three types of suspensions having different particle size distributions were prepared of the hammer-milled, the jet-milled cilostazol crystals and the NanoCrystal® spray-dried powder of cilostazol. In vitro dissolution rate of cilostazol was significantly increased by reducing the particle size. The dissolution curves of the cilostazol suspensions were in good agreement with the simulation based on the Noyes–Whitney equation. The bioavailability of cilostazol after oral administration to dogs was increased with reducing the particle size. While positive food effect on the absorption was observed for the suspensions made of the hammer-milled and the jet-milled crystals, no significant food effect was found for the suspension made of the NanoCrystal® cilostazol spray-dried powder. These results could be qualitatively predicted from the in vitro dissolution data using the bio-relevant media, FaSSIF and FeSSIF. In conclusion, the NanoCrystal® technology is found to be efficient to improve the oral bioavailability of cilostazol and to avoid the food effect on the absorption.

Keywords: Dissolution; Bioavailability; Particle size reduction; Food effect; NanoCrystal; Cilostazol

1. Introduction

Cilostazol is a synthetic antiplatelet agent with vasodialating effect [1]. This drug is approved for a treatment of ischemic symptoms related to peripheral arterial occlusive diseases in Japan and several other countries as Pletaal® tablet, and for a treatment of intermittent claudication in U.S.A. and U.K. as Pletal® tablet [2–5]. Recent study proved that cilostazol is also effective for a prevention of recurrence of cerebral infarction [6]. The molecular weight and melting point of cilostazol are 369.47 and 159.4–160.3 °C, respectively. Cilostazol is a neutral molecule having an aqueous solubility of 3 μg/mL at 25 °C [7]. Octanol–water distribution coefficients ($\log P_{oct}$) of the drug ranged from 2.72 (pH 2.0) to 2.76 (pH 11.0) [7]. An apparent permeability of cilostazol through Caco-2 cell monolayer was found to be 1.92×10^{-5} cm/s [8]. Therefore, according to Biopharmaceutics Classification System (BCS) [9], cilostazol is categorized in Class II (poorly soluble and highly permeable). Fraction dose absorbed of cilostazol from a suspension in 5% ethanol in rats or dogs was found to be 88.0% at 10 mg/kg or 50.7% at 3 mg/kg, respectively, calculated from the recovery of unabsorbed drug in feces [10,11]. The area under the serum concentration–time curve (AUC) of cilostazol for the 50 mg tablet was found to be significantly less (−13%) than that for ethanolic solution in humans. A shorter half-life ($t_{1/2z}$) of cilostazol at the apparent terminal elimination phase after dosing the ethanolic solution (2.5±0.4 h) than that of the tablet (11.0±4.0 h) suggested that the absorption rate constant from the tablet was smaller than the elimination rate constant. These results suggest that the incomplete absorption of cilostazol from the tablet in humans was likely due to the poor dissolution [12].

It is well known that poorly water-soluble drugs often exhibit increased or accelerated absorption when they are

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administered with food [13]. This positive food effect would be attributed to the enhancement of the dissolution rate in the gastrointestinal (GI) tract caused by many factors such as delayed gastric emptying, increased bile secretion, larger volume of the gastric fluid, increased gastric pH (for acidic drugs) and/or increased splanchnic blood flow. In fact, a standard high fat breakfast increased both the rate (+91%) and extent (+24%) of cilostazol absorption after an oral administration of the 100 mg tablet [12], suggesting that the oral bioavailability of cilostazol could be enhanced due to the improvement of dissolution by food.

The dissolution rate of a solid drug can be expressed by the Noyes–Whitney equation [14], and it is also well known that the dissolution rate can be proportionally increased by increasing surface area as a consequence of comminution. Mechanical milling is a common technique to enhance dissolution of poorly water-soluble drugs [15]. Impact mills such as a hammer-mill or fluid energy mills such as a jet-mill are generally used for micronization of active ingredient in pharmaceutical industry [16]. In general, the former produces particles having mean diameters greater than 10 μm and the latter provides particles approximately ten times smaller than the hammer-milled particles. However, it is difficult to reduce particle size in sub-micron region using these dry-mills. NanoCrystal® is an enabling technology to produce sub-micron particles by wet-milling [17–19]. In this technology, materials are grinded with milling beads in water containing steric- and charge-stabilizers to prevent irreversible agglomeration of the resulted sub-micron particles. Anionic surfactants and hydrophilic polymers are used as the charge-stabilizers and the steric-stabilizers, respectively. Significant enhancement of oral bioavailability by this technology was reported for some BCS Class II compounds [17,19]. Elimination of positive food effect was also reported as an advantage of NanoCrystal® [19].

The active ingredient of the commercial formulations is sized with a hammer-mill, resulting in mean particle diameter greater than 10 μm. Oral bioavailability of cilostazol, therefore, is thought to be improved by extensive particle size reduction with a jet-mill or a media-mill (NanoCrystal®). The purpose of the present study was to investigate the effects of particle size on the dissolution rate as well as the rate and the extent of oral absorption of cilostazol. Food effect on the absorption was also investigated.

2. Materials and methods

2.1. Materials

Cilostazol and an internal standard OPC-13012 (6-[4-(1-cyclohexyl-1*H*-tetrazol-5-yl)propoxy]-3,4-dihydro-1-ethyl-2(1*H*)-quinolinone) were synthesized in Otsuka Pharmaceutical Co., Ltd. (Tokushima, Japan). Sodium taurocholate and egg lecithin (biochemistry grade) were obtained from Tokyo Kasei Kogyo Co., Ltd. (Tokyo, Japan) and Kanto Chemical (Tokyo), respectively. All other reagents were analytical grade commercial products.

2.2. Particle size reduction of cilostazol

The hammer-milled cilostazol crystal was prepared with Atomizer AIIW5G (Dalton, Tokyo) and the jet-milled cilostazol crystal was prepared with Super Sonic Jet Mill PJM-100SP (Nippon Pneumatic MFG Co., Ltd. Osaka, Japan). The NanoCrystal® cilostazol spray-dried powder, containing 16.5% of hydroxypropyl cellulose and 0.8% docusate sodium was prepared by spray drying of wet-milled cilostazol dispersion prepared with Dyno®-Mill (type KDL, Glen Mills, Inc., Clifton, NJ, USA). The NanoCrystal® cilostazol spray-dried powder exhibited excellent re-dispersibility in water, the simulated gastric and intestinal fluids in USP. X-ray diffraction analysis indicated that the crystal form of cilostazol was not changed by the milling procedures. No chemical degradation was found by the treatments.

Particle size distributions of the milled cilostazol crystals were determined with a laser diffraction particle size analyzer, SALD-3000J (Shimadzu, Kyoto, Japan), in 0.5% hydroxypropyl methylcellulose aqueous solution as a dispersing medium.

2.3. Preparation of cilostazol suspensions

The NanoCrystal® cilostazol suspension was prepared by dispersing the NanoCrystal® cilostazol spray-dried powder in water at 2.5 mg/mL. The hammer-milled and the jet-milled cilostazol suspensions were prepared with an aqueous solution containing hydroxypropyl cellulose and docusate sodium at the same contents of those in the NanoCrystal® suspension at the same concentrations.

2.4. Preparation of simulated intestinal fluids

The simulated intestinal fluids in the fasted state (FaSSIF) and the fed state (FeSSIF) [20] were utilized as dissolution media in order to predict in vivo dissolution and the food effect on cilostazol absorption. FaSSIF contains 3 mM sodium taurocholate and 0.75 mM lecithin, adjusted at pH 6.5. FeSSIF contains 15 mM sodium taurocholate and 3.75 mM lecithin, adjusted at pH 5.0.

2.5. Solubility measurement

Equilibrium solubility values of cilostazol at 37 °C were determined in water, FaSSIF and FeSSIF. Excess amount of the jet-milled cilostazol crystal was added in each medium in a screw-cap vial. Then, the vials were shaken continuously in a water bath maintained at 37 °C for 24 h. The equilibrated samples were immediately filtered through a 0.2 μm membrane filter, and the filtrate was diluted with appropriate volume of methanol. A 50-μL volume of the sample was analyzed by a reversed-phase HPLC method.

The solubility values of the hammer-milled cilostazol crystal and the NanoCrystal® cilostazol spray-dried powder were estimated from measured values of the jet-milled

crystal using the Ostwald–Freundlich equation [14,21,22] (Eq. (1)).

$$\ln\frac{C_s}{C_{s0}} = \frac{2v\gamma}{rRT} = \frac{2M\gamma}{\rho rRT} \quad (1)$$

where r, v, γ, R and T mean the radius of spherical drug particle, molar volume, interfacial energy, ideal gas constant and absolute temperature, respectively. ρ and M are the density and molecular weight, respectively. C_s is the solubility of the spherical particle, and C_{s0} is the solubility of a flat solid sheet.

2.6. In vitro dissolution profile measurement

In vitro dissolution profiles of the cilostazol suspensions were determined in 900 mL of water, FaSSIF and FeSSIF as dissolution media at 37 °C by USP Apparatus 2 using DT-610 dissolution tester (JASCO, Tokyo). Dissolved cilostazol was quantified from absorbance difference between the wavelengths of 257 nm and 325 nm. Based on the solubility values obtained in the previous section, 5 mg of cilostazol was applied as maximum amount to be solubilized in 900 mL of water. As a preliminary study showed that the dissolution profiles obtained at the speeds of 50 and 200 rpm were equivalent (data not shown), it was fixed at 50 rpm in this research.

2.7. Bioavailability studies in beagle dogs

Cilostazol was orally administered to four beagle dogs (body weight 8–10 kg) as the three types of oral suspensions, the hammer-milled, the jet-milled and the NanoCrystal® suspensions, at 100 mg/body in crossover design. A washout period of 1 week was kept between consecutive dosings. The dogs were fasted for 23 h before dosing and for 10 h post-dosing as a fasted condition. For the fed condition, the dogs were fasted for 22 h until 30 min prior to the dosing, and were then given 170 g of a solid food (CLEA Dog Diet CD-5M containing 24% protein and 9% fat, 635 kcal, CLEA JAPAN, Inc., Tokyo). The complete consumption of the food was ensured every time before dosing. Cilostazol dissolved in 5% DMSO was also intravenously administered to another group of four male beagle dogs (body weight 8–10 kg) at 10 mg/0.2 mL/kg. Dogs were allowed free access to water throughout the experiment. Blood samples (1.5 mL) were collected from a forearm vein with heparinized syringes at 0 (pre-dose), 0.5, 1, 2, 3, 4, 6, 8 and 10 h post-dose for the oral administration and 0 (pre-dose), 0.5, 1, 2, 5, 15, 30, 60 and 120 min post-dose for the intravenous administration. Serum samples were obtained by centrifugation of the blood samples and stored at −20 °C until use. Our investigations were performed after approval by our local ethical committee at Otsuka Pharmaceutical Co. Ltd. and Okayama University and in accordance with “Principles of Laboratory Animal Care” (NIH Publication # 85 - 23).

2.8. Analytical method

2.8.1. Cilostazol in the simulated intestinal fluids

Samples of solubility measurement were introduced onto an HPLC system, consisting of an HPLC pump (Model LC-10A, Shimadzu, Kyoto) and a UV detector (Model SPD-10A, Shimadzu) set at 254 nm. A C18 column (TSK gel ODS-80Ts, 4.6 mm i.d. × 150 mm, Tosoh, Tokyo) was used as an analytical column. A mobile phase containing 0.2% w/v sodium lauryl sulfate and 0.03% v/v phosphoric acid in acetonitrile–methanol–water mixture (3:3:4, v/v/v) was delivered at 1.0 mL/min. Coefficient of variation of standard curve ranged from 0.24% to 1.15% and the correlation coefficient was over 0.999.

2.8.2. Cilostazol in serum

Cilostazol in serum was determined by validated HPLC methods using OPC-13012 as an internal standard. A 200 μL volume of the internal standard solution (1 μg/mL in acetonitrile) was added to a 200 μL volume of the serum sample diluted with blank serum if it was needed. Then it was mixed well with a vortex mixer and stood for 20 min at the room temperature. A 200 μL volume of water was added to the mixture and mixed well with the vortex mixer. The mixture was centrifuged at 1800×g for 10 min, then the supernatant was filtered through a 0.45-μm membrane filter. The samples were applied to an HPLC system, consisting of an HPLC pump (Model Nanospace 3001, Shiseido, Tokyo) and a UV detector (Model Nanospace 3023, Shiseido) set at 254 nm. A 100 μL of the resulting filtrate was injected to the pre-column, TSK pre-column BSA–ODS (4.6 mm i.d. × 10 mm, Tosoh), adjusted at 40 °C and the pre-column was washed with the mobile phase for purification (1:10 diluted phosphate-buffered saline (−)) for 2 min at the flow rate of 500 μL/min, then it was back flushed to the analysis column, Capcell Pak C18 MG S-3 μm (3.0 mm i.d. × 75 mm, Shiseido) adjusted at 40 °C with the mobile phase for analysis (a mixture of acetonitrile–water (2:3 v/v) containing 0.3% v/v of THF) for 5 min at 300 μL/min. The chromatograms were obtained at the detection wavelength of 254 nm. Calibration curves (10–1000 ng/mL, 10 concentrations) and quality control (QC) samples (20, 200 and 1000 ng/mL) were freshly prepared for each analysis. The lower limit of quantification by this method was 10 ng/mL. The linear regression coefficients of the calibration curves ranged from 0.99 to 0.9999. The accuracy and the precision of the QC samples ranged from 92.0% to 118.0% from the nominal values, and 2.3% to 14.5% CV, respectively. Several samples were assayed by using the HPLC system, consisting of the HPLC pump (Model LC-10A, Shimadzu) and the UV detector (Model SPD-10A, Shimadzu) set at 254 nm. TSK precolumn BSA–ODS (4.6 mm i.d. × 35 mm, Tosoh) and YMC AM-302 S-5 μm (4.6 mm i.d. × 150 mm, YMC, Kyoto) were used as a pre-column and an analytical column, respectively. The linear regression coefficients of the calibration curves (10–1000 ng/mL) were higher than 0.9999. The accuracy and the precision of the QC samples ranged from 96.4% to 109.0% from the nominal values, and 0.7% to 5.8% CV, respectively.

2.9. Pharmacokinetic analysis

The highest serum concentration of cilostazol was employed as C_{max}, and the time to reach C_{max} was defined as t_{max}. AUC and the area under the first-moment curve, AUMC, were calculated from 0 to infinity using a linear trapezoidal rule. The serum concentration at time zero (C_0) for intravenous administration was estimated by extrapolation. The mean residence time, MRT, was calculated by AUMC/AUC. The absolute bioavailability (F) was calculated based on Eq. (2).

$$F = \frac{\mathrm{AUC_{oral}}}{\mathrm{AUC_{i.v.}}} \times \frac{\mathrm{Dose_{i.v.}}}{\mathrm{Dose_{oral}}} \tag{2}$$

where $\mathrm{AUC_{oral}}$ and $\mathrm{Dose_{oral}}$ are AUC at infinite time and dose for oral administration of cilostazol. $\mathrm{AUC_{i.v.}}$ and $\mathrm{Dose_{i.v.}}$ mean AUC and dose for intravenous administration of cilostazol.

The in vivo absorption rate of cilostazol was estimated using a numerical deconvolution technique. The mean serum concentration data from the oral administration study were assigned as a response function, while the data from the intravenous administration study were used as a weight function.

2.10. Simulation of in vitro dissolution

In vitro dissolution of the cilostazol suspensions was simulated by the method reported by Hints and Johnson [23], where they expanded a mixing tank model for monodispersed solid drug [24] to polydispersed particles [23,25,26]. Assuming that spherical particles have size fractions $i=1$ to n, that the number of particles dose not change with time and that the diffusion layer thickness at time t is equal to the particle radius at time t, the rate of change in the mass of remaining solid of the particle size fraction i is given by Eq. (3):

$$\frac{\mathrm{d}X_{s_i}}{\mathrm{d}t} = -\frac{3\mathrm{D}X_{0_i}^{2/3}X_{s_i}^{1/3}}{\rho r_{0_i}^2}\left(C_s - \frac{X_{dT}}{V}\right) \tag{3}$$

where X_0 is the initial mass of the drug, X_{si} is the mass of the remaining solid drug at time t for size fraction i. X_{dT} is the total mass of the dissolved drug at time t for the size fractions $i=1$ to n. D and ρ mean the drug diffusivity in aqueous phase and the density of the drug, respectively. r_{0i} is the initial particle radius of particle size for fraction i. C_s and V are the solubility of the drug and fluid volume, respectively. The rate of change in the mass of dissolved drug is given by Eq. (4).

$$\frac{\mathrm{d}X_{d_i}}{\mathrm{d}t} = \frac{3\mathrm{D}X_{0_i}^{2/3}X_{s_i}^{1/3}}{\rho r_{0_i}^2}\left(C_s - \frac{X_{dT}}{V}\right) \tag{4}$$

where X_{d_i} represents the mass of dissolved drug for size i at any time.

The total masses of the remaining solid drug, X_{sT}, and dissolved drug, X_{dT}, at time t for the size fractions $i=1$ to n were described by Eqs. (5) and (6), respectively.

$$X_{sT} = \sum_{i=1}^{n} X_{s_i} \tag{5}$$

$$X_{dT} = \sum_{i=1}^{n} X_{d_i} \tag{6}$$

X_{sT} and X_{dT} were simulated from Eqs. (3)–(6) by the 4th Runge–Kutta numerical integration method using a computer software, Mathematica 5.0 (Wolfram Research Inc., Champaign, IL, USA). The true density of cilostazol was determined with a gas pycnometer, Accupyc 1330 (Micromeritics, Norcross, GA, USA). The diffusivity of cilostazol in water was estimated by a method reported by Hyduk and Laudie [27] using a molar volume estimated by Schroeder's method [28].

2.11. Statistical analysis

Statistical analysis of the effects of particle size and food were performed by Welch's test using PSAG-CP® software (ASmedica, Osaka). P-value less than 0.05 was considered significant.

3. Results and discussion

3.1. Size distribution of milled cilostazol

Particle sizes of the three different types of milled cilostazol were measured and the size distributions are shown in Fig. 1. The median particle diameters of cilostazol in the hammer-milled, the jet-milled and the NanoCrystal® suspensions were found to be 13, 2.4 and 0.22 μm, respectively.

3.2. Solubility

Equilibrium solubility values of the jet-milled cilostazol crystal at 37 °C were determined to be 6.26±0.06, 6.35±0.12 and 12.7±0.2 μg/mL in water, FaSSIF and FeSSIF, respectively. The solubility in FeSSIF superior to that in FaSSIF or water could be attributed not to pH but to micellar solubilization due to higher concentration of sodium taurocholate and lecithin, since cilostazol is a neutral lipophilic compound. The solubility values of the hammer-milled cilostazol crystal and the NanoCrystal® cilostazol spray-dried powder were estimated to be practically identical (0.4% less) and 6% greater than those of the jet-milled cilostazol crystal, respectively. The parameters

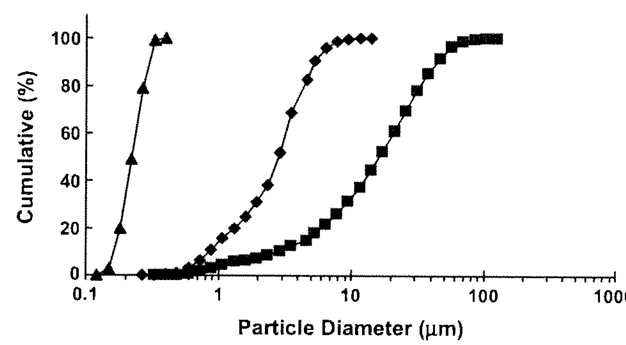

Fig. 1. Particle size distribution of cilostazol suspensions. Keys: ▲, NanoCrystal® spray-dried powder; ♦, jet-milled crystal; ■, hammer-milled crystal.

Table 1
Parameters for dissolution simulation

Parameter	Value
X_0: initial dose	5 mg
C_s: solubility[a]	6.26 μg/mL (in water) 6.35 μg/mL (in FaSSIF) 12.7 μg/mL (in FeSSIF)
D_{eff}: diffusivity	4.04×10^{-4} cm^2/min
ρ: density	1.26 g/cm^3
V: fluid volume	900 mL
Integration step	0.1 min[b] 0.01 min[c]

[a] For the hammer-milled and the NanoCrystal® suspension, these values were multiplied by 0.996 and 1.06, respectively, according to the Ostwald–Freundlich equation.
[b] For the hammer-milled and the jet-milled suspension.
[c] For the NanoCrystal® suspension.

used for this calculation are as follows: M=369.47 g/mol, γ=30 mN/m, ρ=1.26 g/cm^3, R=8.314 × 10^7 g cm^2/s^2/K/mol, T=310 K [22].

3.3. In vitro dissolution rate

In vitro dissolution of cilostazol from the suspension consisting of the hammer-milled, the jet-milled crystals or the NanoCrystal® cilostazol spray-dried powder was investigated in water and the bio-relevant media, FeSSIF and FaSSIF. Solubility of cilostazol in these media is not enough to solubilize clinical doses (50 mg or 100 mg) in 900 mL as shown in the solubility study. Therefore, 5 mg of cilostazol was applied as maximum amount to be solubilized in 900 mL of water, FeSSIF or FaSSIF. Furthermore, the simulation method was also employed to assess the dissolution of cilostazol from the suspensions. The parameters used for the simulation are listed in Table 1. Results shown in Fig. 1 were used as data of particle size distribution. Solubility values obtained in the previous section were also used for the simulation.

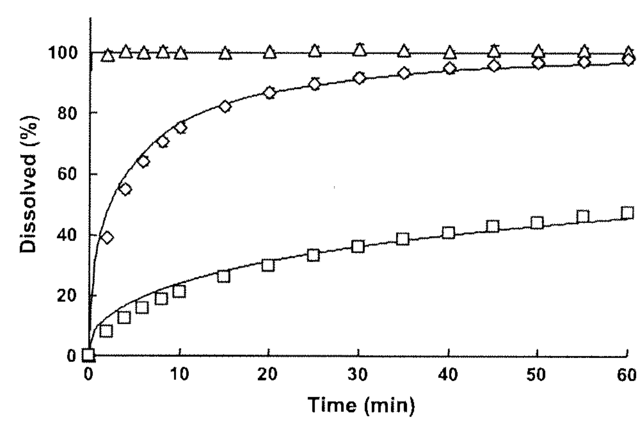

Fig. 2. In vitro dissolution profiles of cilostazol from the suspensions in water at 37 °C. Dissolution study was performed at 50 rpm following USP Apparatus 2. About 5 mg of cilostazol was applied in 900 mL water. Results are expressed as the mean with the bar showing S.D. values of six experiments and simulated curves (solid lines). Keys: △, NanoCrystal® spray-dried powder; ◇, jet-milled crystal; □, hammer-milled crystal.

Table 2
Simulated 50% dissolution time ($T_{50\%}$, min) of cilostazol suspensions at 37 °C

Formulation	Dissolution medium		
	Water	FaSSIF	FeSSIF
NanoCrystal®	0.016	0.016	0.0068
Jet-milled	2.3	2.3	0.97
Hammer-milled	82	80	32

Calculated from simulation results.

3.3.1. Dissolution in water

The dissolution profiles of the cilostazol suspensions in water are shown in Fig. 2. The dissolution rate of cilostazol from the suspensions was clearly affected by the particle size of the active ingredient. From the hammer-milled suspension, only 45% of applied cilostazol was dissolved in 60 min. In the case of the jet-milled suspension, more than 80% of cilostazol was dissolved within 15 min and the dissolved fraction reached 95% in 60 min. In contrast, the dissolution of cilostazol from the NanoCrystal® suspension was completed immediately. As sub-micron particles such as NanoCrystal® cilostazol can easily pass through a line filter, it is possible to overestimate the dissolution rate. A high-speed centrifugation method [29], therefore, was attempted to separate the solid particles (data not shown), but it was not appropriate because of considerably long time to separate them and the difficulty in controlling temperature during the centrifugation. Although the possible overestimation cannot be excluded, the simulated line agreed with the actual data very well, indicating the extremely rapid dissolution of the NanoCrystal® cilostazol suspension. Visual observation of immediate disappearance of turbidity in the dissolution vessel also suggested that the solid particles passed through the filter could be ignored.

The excellent agreement between the observed data and the simulated dissolution curves suggested that the dissolution of cilostazol from the suspensions followed the Noyes–Whitney equation and that the model with the assumptions and the parameters used were appropriate. The results shown in Fig. 2

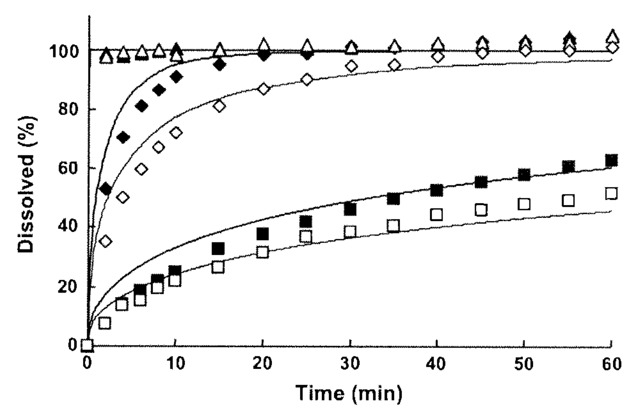

Fig. 3. In vitro dissolution profiles of cilostazol from the suspensions in the bio-relevant media at 37 °C. Dissolution study was performed at 50 rpm following USP Apparatus 2. About 5 mg of cilostazol was applied in 900 mL water. Results are expressed as the mean of two experiments and simulated curves (fine lines for FaSSIF and bold lines for FeSSIF). Keys: In FaSSIF: △, NanoCrystal® spray-dried powder; ◇, jet-milled crystal; □, hammer-milled crystal; In FeSSIF: ▲, NanoCrystal® spray-dried powder; ♦, jet-milled crystal; ■, hammer-milled crystal.

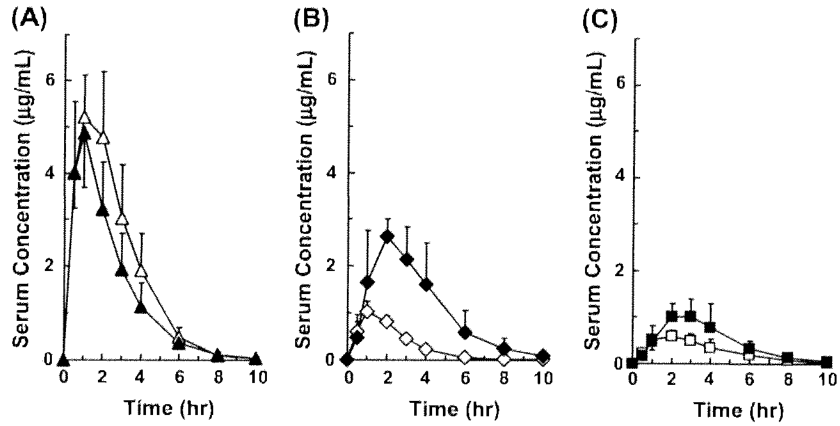

Fig. 4. Serum concentration–time profiles of cilostazol after oral administration of the suspensions at a dose of 100 mg/body in beagle dogs. Results are expressed as the mean with the bar showing S.D. values of four experiments. Keys: (A) △, NanoCrystal® suspension (fasted); ▲, NanoCrystal® suspension (fed); (B) ◇, jet-milled suspension (fasted); ♦, jet-milled suspension (fed); (C) □, hammer-milled suspension (fasted); ■, hammer-milled suspension (fed).

were much better than those obtained when cilostazol was treated as monodispersed particles with a mean particle size (data not shown).

Assuming smooth spherical particles, specific surface area is inversely proportional to particle size. Accordingly, the specific surface area of the jet-milled cilostazol crystal and the cilostazol crystal in the NanoCrystal® spray-dried powder were calculated as 3.2- and 28-fold greater than that of the hammer-milled crystal, treated as spherical polydispersed particles having size distribution shown in Fig. 1. Based on the simulated curves, 50% dissolution times ($T_{50\%}$) of the hammer-milled, the jet-milled and the NanoCrystal® suspensions were calculated to be 82 min, 2.3 min and 0.016 min, respectively (Table 2), indicating that the dissolution rates of the jet-milled and the NanoCrystal® suspensions would be 36-fold and 5100-fold greater than that of the hammer-milled suspension, respectively.

3.3.2. Dissolution in bio-relevant media

In order to predict the food effect on the absorption of cilostazol from the suspensions, the dissolution was investigated in the bio-relevant media, FaSSIF and FeSSIF (Fig. 3), because food effects on the extent of absorption of BCS Class II drugs such as danazol and atovaquone were well correlated with the FeSSIF/FaSSIF ratio of the in vitro dissolved amount [20,30–32]. The data obtained from two vessels for each suspension were superimposed, and the simulated lines agreed fairly well with the observed data. The dissolution rates of cilostazol in FaSSIF were similar to, and those in FeSSIF were faster than those obtained in water for all the suspensions. Simulation study showed that the $T_{50\%}$ values for the hammer-milled, the jet-milled and the NanoCrystal® cilostazol suspensions were 80, 2.3 and 0.016 min in FaSSIF, and 32, 0.97 and 0.0068 min in FeSSIF, respectively (Table 2). Although the FeSSIF/FaSSIF ratio of $T_{50\%}$ for each suspension was around 0.4 regardless of the difference in particle size, the difference in the absolute value of $T_{50\%}$ between FeSSIF and FaSSIF became much smaller as the particle size reduced. Especially, for the NanoCrystal® suspension, it can practically be neglected. These results suggest that the food effect can be avoided by the enhancement of dissolution rate due to the reduction of particle size.

3.4. Bioavailability study in dogs

The serum concentration–time profiles and the pharmacokinetic parameters of cilostazol resulted from the oral admin-

Table 3
Pharmacokinetic parameters

Food condition	Parameter	Intravenous administration (10 mg/kg)	NanoCrystal® (100 mg/body)	Jet-milled (100 mg/body)	Hammer-milled (100 mg/body)
Fasted	C_{max} (ng/mL)	–	5371±1173***	1029±218	582±154
	AUC (ng h/mL)	17584±7556	17832±4994**	2875±587	2722±803
	t_{max} (h)	–	1.3±0.5	1.0±0.0	1.8±0.5
	MRT (h)	1.3±0.4	2.4±0.2**	2.3±0.2**	3.6±0.9
	F	1	0.86±0.29	0.15±0.04	0.14±0.05
Fed	C_{max} (ng/mL)	–	4872±1112***	2901±314**	1152±221
	AUC (ng h/mL)	–	13589±3895**	10669±3417*	4694±1612
	t_{max} (h)	–	1.0±0.0*	1.0±0.0	2.5±1.3
	MRT (h)	–	2.3±0.3*	3.4±0.8	3.7±0.6
	F	–	0.67±0.22	0.53±0.21	0.23±0.09

Results are expressed as the mean±S.D. of four experiments. ***$P<0.001$; **$P<0.01$; *$P<0.05$, compared to the corresponding parameters of the hammer-milled suspension.

CL_{total}, k_{el} and Vd_{ss} for the intravenous administration were calculated to be 5.4±1.8 L/h, 0.79±0.20 h^{-1} and 6.5±0.7 L, respectively.

Table 4
Fed/fasted ratio on pharmacokinetic parameters

Parameter	NanoCrystal® (100 mg/body)	Jet-milled (100 mg/body)	Hammer-milled (100 mg/body)
C_{max}	0.91±0.13	2.9±0.5***	2.0±0.3**
AUC	0.76±0.04	3.7±0.7**	1.8±0.6
t_{max}	0.88±0.25	1.8±0.5*	1.0±0.0
MRT	0.95±0.13	1.5±0.4*	1.1±0.4
F	0.78±0.04	3.6±0.7	1.8±0.6

Results are expressed as the mean±S.D. of four experiments. ***P<0.001; **P<0.01; *P<0.05, statistically significant difference between the fed state parameters and the fasted state parameters.

istrations of the suspensions in beagle dogs are presented in Fig. 4 and Table 3, respectively. To estimate the food effect, the fed/fasted ratios on the pharmacokinetic parameters were also listed in Table 4.

In the fasted condition, compared with the hammer-milled cilostazol suspension, the absorption of cilostazol was not improved so much by dosing the jet-milled suspension as shown in Fig. 4 and Table 3. On the other hand, the NanoCrystal® suspension significantly increased C_{max} and AUC of cilostazol 9.2 (P<0.001) and 6.7 times (P<0.05), respectively, resulting in 86% of the absolute bioavailability. These results indicated that the extensive reduction of particle size could lead to the improvement of bioavailability of cilostazol. Although it was expected that the jet-milled suspension also had improved the bioavailability of cilostazol based on the results of the in vitro dissolution study (Figs. 2 and 3), the absorption was not changed so much, which might be attributed to the difference in dissolution behavior between in vitro and in vivo.

In the fed condition, the C_{max} values of the hammer-milled and the jet-milled suspension were increased 2.0-fold (P<0.01) and 2.9-fold (P<0.001), respectively, and the AUC values were increased 1.8-fold (not statistically significant) and 3.7-fold (P<0.01), respectively (Table 3). These results clearly indicate the positive food effects on the rate and extent of cilostazol absorption for the hammer-milled and the jet-milled suspension. On the other hand, in the case of the NanoCrystal® suspension, the fed/fasted ratios of C_{max} and the AUC values were 0.91-fold (not statistically significant) and 0.76-fold (not statistically significant), respectively, suggesting slightly negative food effects for the NanoCrystal® suspension.

These results suggest that the in vivo dissolution of cilostazol from the hammer-milled and the jet-milled suspension was improved by the food intake, which could be supported by the absorption rate–time profiles of cilostazol after oral dosing (Fig. 5). As cilostazol is classified into Class II of BCS, the absorption could be rate-limited by the dissolution process. Therefore, the profiles shown in Fig. 5 could reflect the in vivo dissolution behavior as well and clearly indicate that the food intake enhanced and prolonged the dissolution and absorption of cilostazol in the cases of the hammer-milled and the jet-milled suspensions. The prolongation of the dissolution, which is also suggested by the values of MRT (Table 3), might be ascribed to the improvement of dissolution by the food and/or bile and to the delay of gastric emptying by the food [33]. On the other hand, the dissolution and absorption of cilostazol from the NanoCrystal® suspension were not enhanced and not prolonged by the foods (Table 3 and Fig. 5) because the dissolution might be maximally improved by the size reduction and the drug might partially adsorb to the ingested food. Fig. 5 also shows that initial dissolution/absorption rates in 1 h after administration for the hammer-milled, the jet-milled and the NanoCrystal® cilostazol suspensions were 4.9%, 10.1% and 51.7% in the fasted condition, 4.6%, 14.2% and 50.5% in the fed condition, respectively. These results clearly indicated that the smaller particle resulted in the faster dissolution/absorption. However, it was also shown that food did not enhance the apparent initial absorption rate, which might be explained by the delay of gastric emptying by the food intake [33]. As gastric emptying is another possible rate-limiting step for the absorption, the retardation of gastric emptying could cancel out the potency of the dissolution enhancement.

The particle size reduction certainly enhanced the in vitro dissolution rate and the in vivo dissolution/absorption of cilostazol, but the relationship in the enhancement of dissolution between in vitro and in vivo was not necessarily

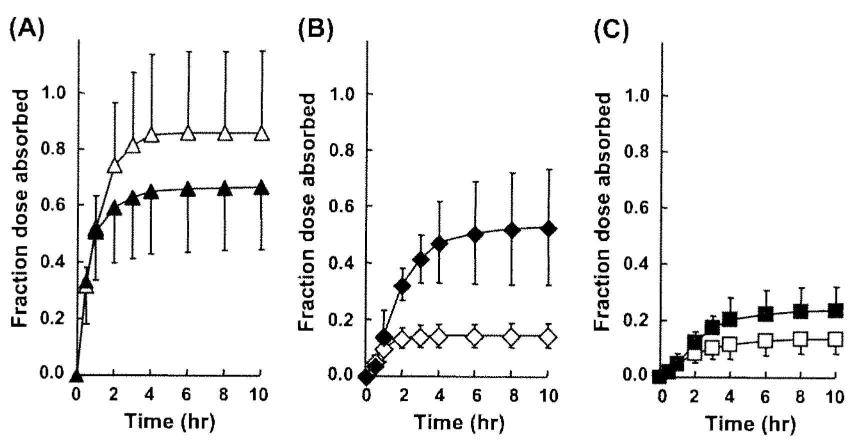

Fig. 5. Absorption rate–time profiles of cilostazol after oral administration of suspensions at a dose of 100 mg/body in beagle dogs. Results are expressed as the mean with the bar showing S.D. values of four experiments. Keys: (A) △, NanoCrystal® suspension (fasted); ▲, NanoCrystal® suspension (fed); (B) ◇, jet-milled suspension (fasted); ◆, jet-milled suspension (fed); (C) □, hammer-milled suspension (fasted); ■, hammer-milled suspension (fed).

quantitative. The initial dissolution/absorption rates described above, based on the results shown in Fig. 5, are also obviously larger than the cumulative dissolved amounts for 1 h in the in vitro dissolution study (Fig. 3). It would be difficult to find out the quantitative relationship between in vivo dissolution and in vitro dissolution by a simple dissolution study as employed in the present study, because such a dissolution study is not able to reflect the maintenance and enhancement of the dissolution, which could be ascribed to the sink condition that might be kept to some extent by the sequential absorption right after dissolution in vivo.

In the case of the hammer-milled suspension, which might correspond to the commercial tablet in human study, the bioavailability of cilostazol was enhanced around twice by the food intake, which is in good agreement with the human data where C_{max} and AUC of cilostazol after dosing the commercial 100 mg tablet were 1.7- to 1.9-fold and 1.2- to 1.3-fold greater, respectively, in the fed condition than those in the fasted condition [12]. This food effect would be related with the FeSSIF/FaSSIF ratio in the dissolution rate and/or solubility. This might also be the case with the jet-milled suspension. Although the food effect was a little more significant for the jet-milled cilostazol than that for the hammer-milled one, the enhancement ratios in C_{max} and AUC observed for these suspensions were still comparable to the FeSSIF/FaSSIF ratio in the dissolution rate and/or solubility. On the other hand, the food intake did not increase the bioavailability of the NanoCrystal® cilostazol suspension. Taken all together, the food effect could be independent of the solubility, because the solubility and the FeSSIF/FaSSIF ratio in the solubility were almost same for all the three suspensions. The improvement of dissolution rate should be responsible for the improved bioavailability of cilostazol in the cases of the jet-milled and the hammer-milled suspensions. The reason why no food effect was observed for the NanoCrystal® cilostazol suspension might be that the dissolution rate of NanoCrystal® cilostazol is fast enough even under the fasted condition, where the absorption might be permeability-limited. Therefore, the further increase in the dissolution rate would not contribute to the improvement of the absorption.

4. Conclusion

In the present study, it was demonstrated that the in vitro dissolution rate of cilostazol from aqueous suspension was improved by milling, which was described by the Noyes–Whitney equation. The bioavailability of cilostazol was increased by the reduction of particle size, but remarkable enhancements with minimum food effect were observed for the NanoCrystal® cilostazol suspension, while moderate enhancement of bioavailability and significant food effect were seen in the absorption for the jet-milled suspension, compared to the suspension made of the conventional hammer-milled crystal. These findings clearly indicate that the bioavailability of cilostazol can be maximized and the food effect can be avoided by the NanoCrystal® technology for a robust formulation.

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EXHIBIT 3

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NANOSUSPENSION: AN ATTEMPT TO ENHANCE BIOAVAILABILITY OF POORLY SOLUBLE DRUGS

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ABSTRACT

Most of the new chemical entities coming out from High-throughput screening in drug discovery process are failing due to their poor solubility in the water. Poorly water-soluble drugs show many problems in formulating them in conventional dosage forms. One of the critical problems associated with poorly soluble drugs is too low bioavailability. The problem is even more complex for drugs belonging to BCS CLASS II category, as they are poorly soluble in both aqueous and organic media, and for those drugs having a log P value of 2. There are number of formulation approaches to resolve the problems of low solubility and low bioavailability. These techniques for solubility enhancement have some limitations and hence have limited utility in solubility enhancement. Nanotechnology can be used to resolve the problems associated with these conventional approaches for solubility and bioavailability enhancement. Nanotechnology is defined as the science and engineering carried out in the nanoscale that is 10^{-9} meters. The present article describes the details about nanosuspensions. Nanosuspensions consist of the pure poorly water-soluble drug without any matrix material suspended in dispersion. The review article includes the methods of preparation with their advantages and disadvantages, characterization and evaluation parameters and pharmaceutical applications. A nanosuspension not only solves the problems of poor solubility and bioavailability but also alters the pharmacokinetics of drug and thus improves drug safety and efficacy.

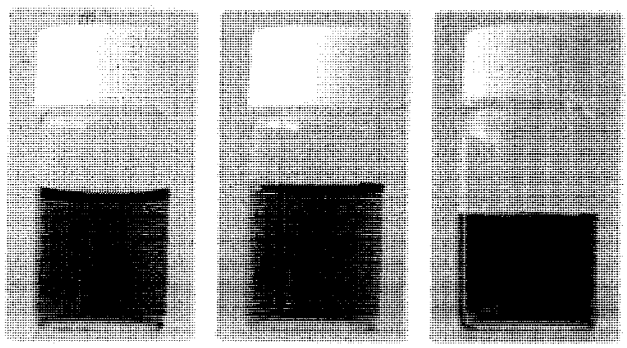
INTRODUCTION: Most of the new chemical entities (about 40%) coming out from High-throughput screening in drug discovery process are failing due to their poor solubility in the water [1]. As per a recent report [2], 46% of the total New Drug Applications (NDA) filed between 1995 and 2002 were BCS class IV, while only 9% were BCS class I drugs, revealing that a majority of the approved new drugs were water insoluble Because of their poor solubility it will become more complicated to incorporate them into the conventional dosage forms and thus decreasing the bioavailability of the drugs [3].

The problem is even more complex for drugs such as Glibenclamide (belonging to BCS CLASS II) as classified by BCS System [4] as they are poorly soluble in both aqueous and organic media, and for those drugs having a log P value of 2. For class II drugs, the rate limiting factor in their intestinal absorption is dissolution/solubility and thus the performance of these drugs is dissolution rate-limited and is affected by the fed/fasted state of the patient. Dissolution rates of sparingly soluble drugs are greatly affected by the shape as well as the particle size of the drug. Therefore decrease in particle size results in an increase in dissolution rate [5]. There are number of formulation approaches that can be used to resolve the problems associated with the low solubility and low bioavailability of these class II drugs. Some of the approaches to increase solubility include micronization [6], solubilisation using co-solvents, use of permeation enhancers, oily solutions, surfactant dispersions [6], salt formation [7] and precipitation techniques [8-9].

Most of these techniques for solubility enhancement have advantages as well as some limitations and hence have limited utility in solubility enhancement. Other techniques used for solubility enhancement like microspheres, emulsions, microemulsions [10], liposomes [11], super critical processing, solid-dispersions [12] and inclusion complexes using Cyclodextrins [13] show reasonable success but they lack in universal applicability to all drugs. These techniques are not applicable to the drugs, which are not soluble in both aqueous and organic Media.

However, there still remains an unmet need to equip the pharmaceutical industry with particle engineering technologies capable of formulating the poorly soluble drugs to improve their efficacy and to optimize therapy with respect to pharmacoeconomics. One such novel technology is nanosuspension technology. Nanosuspensions are sub-micron colloidal dispersions of nanosized drug particles stabilized by surfactants [14]. Nanosuspensions consist of the poorly water-soluble drug without any matrix material suspended in dispersion [15]. These can be used to enhance the solubility of drugs that are poorly soluble in aqueous as well as lipid media. As a result of increased solubility, the rate of flooding of the active compound increases and the maximum plasma level is reached faster.

This is one of the unique advantages that it has over other approaches for enhancing solubility. This approach is useful for molecules with poor solubility, poor permeability or both, which poses a significant challenge for the formulators. The reduced particle size renders the possibility of intravenous administration of poorly soluble drugs without any blockade of the blood capillaries. The suspensions can also be lyophilised and into a solid matrix. Apart from these advantages it is also having the advantages of liquid formulations over others. In the present review we are mainly focussing on the different methods of preparation, critical parameters and evaluation of the nanosuspension. Fig. 1 shows some of the nanosuspensions [16].



A- Gold nanosuspension in water, **B** -Silver nanosuspension in water, **C** -VOPc (vanadyl phthalocyanine) nanosuspension in water

FIGURE 1: FEW TYPES OF NANOSUSPENSIONS.

Nanosuspensions differ from nanoparticles [17] which are polymeric colloidal carriers of drugs (Nanospheres and nanocapsules), and from solid-lipid nanoparticles [18] (SLN), which are lipidic carriers of drug. The potential benefits of nanoparticles over conventional technologies are described in **Table 1** [19].

TABLE 1: POTENTIAL BENEFITS OF NANOSUSPENSION TECHNOLOGY

ROUTE OF ADMINISTRATION	POTENTIAL BENEFITS
Oral	• Rapid dissolution and • High bioavailability • Reduced fed/fasted ratio
Intravenous (I.V)	• Tissue targeting • Rapid dissolution • Longer duration of retention in systemic circulation
Ocular	• Higher bioavailability • Less irritation • More consistent dosing
Inhalation	• Higher bioavailability • More consistent dosing
Subcutaneous/ intramuscular	• Higher bioavailability • Rapid onset • Reduced tissue irritation

Preparation of Nanosuspensions: Preparation of nanosuspensions were reported to be a more cost effective and technically more simpler alternative than liposomes and other conventional colloidal drug carriers, particularly for poorly soluble drugs and yield a physically more stable product. The simplest method of preparation of nanosuspensions is micronization by colloid or jet milling [20], which improves the dissolution rate but is not having any effect on saturation solubility. Nanosuspension engineering processes currently used are preparation by precipitation, high pressure homogenization, emulsion and milling techniques. These techniques and the obtained compounds are summarized in **Table 2** and are briefly described in the following sections. Mainly there are two methods for preparation of nanosuspensions. The conventional methods of precipitation are called 'Bottom Up technology'. The 'Top Down Technologies' are the disintegration methods and are preferred over the precipitation methods. These include Media Milling (Nanocrystals), High Pressure Homogenization in water (Dissocubes), High Pressure Homogenization in nonaqueous media (Nanopure) and combination of Precipitation and High-Pressure Homogenization (Nanoedege). Few other techniques used for preparing nanosuspensions are emulsion as templates, microemulsion as templates etc.

- **Precipitation:** The most common method of precipitation used is anti solvent addition method in which the drug is dissolved in an organic solvent and this solution is mixed with a miscible antisolvent. Mixing processes vary considerably. Precipitation has also been coupled with high shear processing. The NANOEDGE process (is a registered trademark of Baxter International Inc. and its subsidiaries) relies on the precipitation of friable materials for subsequent fragmentation under conditions of high shear and/or thermal energy [32].

TABLE 2: SUMMARY OF THE NANOSUSPENSION FORMATION TECHNOLOGIES

Technology	Advantage	Disadvantage	Drug
Precipitation	Simple process. Ease of scale up. Economical production.	Growing of crystals needs to be limit by surfactant addition. Drug must be soluble at least in one solvent.	Carbamazepine [8] Cyclosporine [21] Griseofulvin [22]
Emulsion/Microemulsion template	High drug solubilization. Long shelf life. Ease of manufacture.	Use of high amount of surfactant and stabilizers. Use of hazardous solvent in production.	Breviscapine [23] Griseofulvin [24]
High pressure Homogenization	Applicable to most of the drugs Very dilute as well as highly concentrate nanosuspension can be prepared. Aseptic production possible.	High number of homogenization cycles. Drug should be in micronized state. Possible contamination could occur from metal ions coming off from the walls.	Albendazole [25] Aphidicolin [26] Azithromycin [27] Fenofibrate [28]
Milling methods			
• Media milling	Applicable to the drugs that are poorly soluble in both aqueous and organic media. Little batch to batch variation. High flexibility in handling large quantities of drugs.	Time consuming. Difficult to scale up. Prolonged milling may induce the formation of amorphous & instability.	Cilostazol [29] Danazol [3] Naproxen [3]
• Dry Co-grinding	Easy process and no organic solvent required. Require short grinding time.	Generation of residue of milling media.	Clarithromycin [30] Glibenclamide [31]

This is accomplished by a combination of rapid precipitation and high-pressure homogenization. Rapid addition of a drug solution to an antisolvent leads to sudden super saturation of the mixed solution, and generation of fine crystalline or amorphous solids. Precipitation of an amorphous material may be favored at high super saturation when the solubility of the amorphous state is exceeded. The success of drug nanosuspensions prepared by precipitation techniques has been reported in some journals [32-33].

- **Lipid Emulsion/Microemulsion Template:** Lipid emulsions as templates are applicable for drugs that are soluble in either volatile organic solvents or partially water miscible solvents. In this method the drug will be dissolved in the suitable organic solvent and then emulsified in aqueous phase using suitable surfactants. Then the organic solvent will be slowly evaporated under reduced pressure to form drug particles precipitating in the aqueous phase forming the aqueous suspension of the drug in the required particle size. Then the suspension formed can be diluted suitably to get nanosuspensions [34]. Moreover, microemulsions as templates can produce nanosuspensions. Microemulsions are thermodynamically stable and isotropically clear dispersions of two immiscible liquids such as oil and

water stabilized by an interfacial film of surfactant and co-surfactant. The drug can be either loaded into the internal phase or the pre-formed microemulsion can be saturated with the drug by intimate mixing. Suitable dilution of the microemulsion yields the drug nanosuspension [34]. An example of this technique is the griseofulvin nanosuspension which is prepared by the microemulsion [34]. The advantages of lipid emulsions as templates for nanosuspension formation are that they easy to produce by controlling the emulsion droplet and easy for scale-up. However, the use of organic solvents affects the environment and large amounts of surfactant or stabilizer are required.

- **High Pressure Homogenization:** It is the most widely used method for the preparation of the nanosuspensions of many poorly water soluble drugs [35-37]. Different methods developed based on this principle for preparation of nanosuspensions are *Dissocubes, Nanopure, Nanoedge, Nanojet technology.* In the high pressure homogenization method, the suspension of a drug and surfactant is forced under pressure through a nanosized aperture valve of a high pressure homogenizer.

The principle of this method is based on cavitation in the aqueous phase. The particles cavitations forces are sufficiently high to convert the drug microparticles into nanoparticles. The concern with this method is the need for small sample particles before loading and the fact that many cycles of homogenization are required [38-39]. Figure 2 gives the schematic representation of the high-pressure homogenization process

- DissoCubes technology is an example of this technology developed by R.H. Müller using a piston-gap-type high pressure homogenizer, which was recently released as a patent owned by SkyePharm plc [34]. Scholer *et al.* prepared atovaquone nanosuspensions using this technique.
- Nanopure is suspensions homogenized in water-free media or water mixtures.
- Nanoedge is combination of precipitation and homogenization techniques resulting in smaller particle size and better stability in a shorter time.
- *Nanojet technology*, also called as opposite stream, uses a chamber where a stream of suspension is divided into two or more parts, which colloid with each other at high pressure.

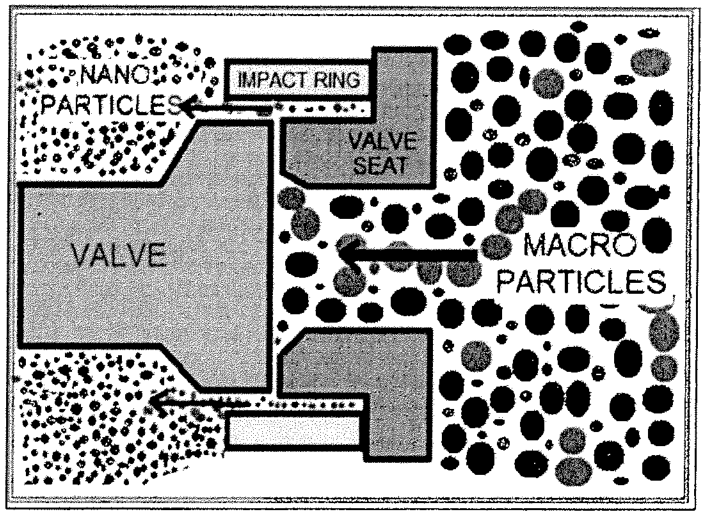


FIGURE 2: SCHEMATIC CARTOON OF THE HIGH-PRESSURE HOMOGENIZATION PROCESS

- **Milling Techniques:**
  - **Media milling:** Media milling is a further technique used to prepare nanosuspensions [24, 40]. This patent-protected technology was developed by Liversidge et al. [41]. Formerly, the technology was owned by the company NanoSystems but recently it has been acquired by Elan Drug Delivery. In this technique, the nanosuspensions are produced using high-shear media mills or pearl mills. The media mill consists of a milling chamber, a milling shaft and a recirculation chamber. The drug nanoparticles are obtained by subjecting the drug to media milling. High energy and shear

forces generated as a result of impaction of the milling media with the drug provide the necessary energy input to disintegrate the microparticulate drug into nanosized particles. The milling medium is usually composed of glass, zirconium oxide or highly cross-linked polystyrene resin. In batch mode, the time required to obtain dispersions with unimodal distribution profiles and mean diameters <200nm is 30–60 min. In the media milling process, the milling chamber is charged with the milling media, water or suitable buffer, drug and stabilizer. Then milling media or pearls are rotated at a very high shear rate.

- **Dry Co-Grinding:** Recently, nanosuspensions can be obtained by dry milling techniques. Successful work in preparing stable nanosuspensions using dry-grinding of poorly soluble drugs with soluble polymers and copolymers after dispersing in a liquid media has been reported [42]. Itoh *et al* [35] reported the colloidal particles formation of many poorly water soluble drugs; griseofulvin, glibenclamide and nifedipine obtained by grinding with polyvinylpyrrolidone (PVP) and sodium dodecylsulfate (SDS).

Many soluble polymers and co-polymers such as PVP, polyethylene glycol (PEG), hydroxypropyl methylcellulose (HPMC) and cyclodextrin derivatives have been used [43]. Physicochemical properties and dissolution of poorly water soluble drugs were improved by co-grinding because of an improvement in the surface polarity and transformation from a crystalline to an amorphous drug [44]. Dry co-grinding can be carried out easily and economically and can be conducted without organic solvents. The co-grinding technique can reduce particles to the submicron level and a stable. Table 3 shows some drugs and their status in market.

TABLE 3: SOME DRUGS AND THEIR STATUS IN MARKET

Drug	Category	Route of Administration	Status
Fenofibrate	Anticancer	Oral	Phase I
Rapamuane	Antiemetic	Oral	Marketed
Emend	Antiasthamatic	Oral	Marketed
Thymectacin	Antidiabetic	I.V.	Phase I/II
Silver	Eczema, Atopic dermatitis	Topical	Phase I
Busulfan	Hypolidemic	Intrathecal	Phase I
Paclitaxel	Anticancer	I. V.	Phase IV
Insulin	Antidiabetic	Oral	Phase I
Budesonide	Anticancer	Pulmonary	Phase I

Physical, Chemical and Biological Properties of Nanosuspensions: Nanosuspension formulation increases the saturation solubility as well as dissolution rate. Basically the saturation solubility is a compound specific constant which is temperature dependent. The saturation solubility also depends on the polymorphism of the drug as different polymorphs have different solubilities. It is also dependent on the particle size. This size-depend encycomes only into effect for particles having a size below approximately 1 µm. Another marked property is the adhesiveness generally described for nanoparticles [45].

As the particle size decreases the adhesive properties of the particles will be improved and thus improved oral delivery of poorly soluble drugs. Improved bioavailability, improved dose proportionality, reduced fed/fasted variability, reduced inter-subject variability and enhanced absorption rate (both human and animal data) [46] are some of the main effects observed on oral administration. These data have been acquired *in vivo* in animals but also in humans as reported by the company Nano Systems. A drastically remarkable report is that of the increase in bioavailability for danazole from 5 % (as macrosuspension) to 82% (as nanosuspension) [46]. The application of high

pressures during the production of nanosuspensions was found to promote the amorphous state [47]. The degree of particle fineness and the fraction of amorphous particles in the nanosuspensions were found to be dependent on production pressure number of cycles of homogenisation and hardness of drug. The increase in the amorphous fraction leads to a further increase of the saturation solubility. The homogenization process (giving uniform particle size) was able to overcome Ostwald ripening [48] which means physical long-term stability as an aqueous suspension [49].

In oral drug administration, the bioavailability mainly depends upon the solubility of the drug, highly active compounds have failed in the past because their poor solubility has limited *in vivo* absorption and did not lead to effective therapeutic concentrations. As an example, Atovaquone is given orally three times 750 mg daily, because of the low absorption of only 10–15%. Oral administration of nanosuspensions can overcome this problem because of the high adhesiveness of drug particles sticking on biological surfaces and prolonging the absorption time.

Evaluation of Nanosuspensions [50-51]: The characterisation of the nanosuspensions is also similar to that of the suspensions such as colour, odour, presence of impurities and other important characteristics as mentioned below.

- ***In-Vitro* Evaluations:**
  - **Particle size and size distribution**
  - **Particle charge (Zeta Potential)**
  - **Crystalline state and morphology**
  - **Saturation solubility and dissolution velocity**
  - **Stability**
- ***In-vivo* evaluation:**
- ***In-Vitro* Evaluations:**

- **Particle size and size distribution:** It is the most important parameter in the evaluation of the suspensions as it is having the direct effect on the solubility and dissolution rate and the physical stability of the formulation. The mean particle size and the width of particle size can be determined by Photon Correlation Spectroscopy (PCS) [52], laser diffraction and coulter current multisizer. Particle size and polydispersity index (PI) governs the saturation solubility, dissolution velocity and biological performance. PCS measures the particle size in the range of 3nm-3 µm only. PI governs the physical stability of nanosuspension and should be as low as possible for long-term stability (Should be close to zero). LD measures volume size distribution and measures particles ranging from 0.05- 80µm upto 2000µm. Atomic Force Microscopy is used for visualization of particle shape [53]. For IV use, particles should be less than 5 µm, considering that the smallest size of the capillaries is 5-6 µm and hence a higher particle size can lead to capillary blockade and embolism.
- **Particle charge (Zeta Potential):** The particle charge is of importance in the study of the stability of the suspensions. Usually the zeta potential of more than ±40mV will be considered to be required for the stabilisation of the dispersions. For electrostatically stabilized nanosuspension a minimum zeta potential of ±30mV is required and in case of combined steric and electrostatic stabilization it should be a minimum of ±20mV of zeta potential is required.
- **Crystalline Sate and Particle Morphology:** It is of importance as there are chances of the polymorphism during the storage of the nanosuspensions. Hence it is necessary to study the crystal morphology of the drug in suspension. Differential Scanning Calorimetry

(DSC) is most commonly used for such studies [54]. When nanosuspensions are prepared drug particles may get converted to amorphous form hence it is essential to measure the extent of amorphous drug generated during the production of nanosuspensions. The X-Ray Diffraction (XRD) is commonly used for determining change in crystallinity and the extent of the amorphous form of drug [55].

- **Saturation solubility and Dissolution Velocity:** The main advantage associated with the nanosuspensions is improved saturation solubility as well as dissolution velocity. These are studied in different physiological solutions at different pH. Kelvin equation and the Ostwald-Freundlich equations can explain increase in saturation solubility. Determination of these parameters is useful to assess *in vivo* performance of the formulation.

- **Stability of Nanosuspensions:** Stability of the suspensions is dependent on the particle size. As the particle size reduces to the nanosize the surface energy of the particles will be increased and they tend to agglomerate. So stabilizers are used which will decrease the chances of Ostwald ripening and improving the stability of the suspension by providing a steric or ionic barrier. Typical examples of stabilizers used in nanosuspensions are cellulosics, poloxamer, polysorbates, lecithin, polyoleate and povidones. Lecithin may be preferred in developing parenteral nanosuspensions [40].
- ***In vivo* evaluation:** The *in vivo* evaluation of the nanosuspensions is specific to drug and route of administration. Most commonly the formulation was given by required route of administration and the plasma drug levels were estimated using HPLC-UV visible Spectrophotometry. Other parameters which are generally evaluated *in vivo* are
  - Surface hydrophilicity/hydrophobicity (determines interaction with cells prior to phagocytosis)
  - Adhesion properties
  - Interaction with body proteins

APPLICATIONS: Formulating the drug as nanosuspensions increases the saturable concentration, dissolution rate as well as bioavailability of the drug. These nanosuspensions are having application in different routes of administrations like oral, parenteral, topical, ophthalmic, mucoadhesive, pulmonary and targeted drug delivery. Oral administration of nanosuspensions is a drug delivery strategy, not only to improve bioavailability, but also to target gastrointestinal bacterial and parasitic infections because of improved adhesion properties. Nanosuspension technology is considered as suitable new colon delivery systems for the treatment of colon cancer, helminth infections, gastrointestinal inflammation or GIT associated diseases like sprue (zoeliaki).

Infections like tuberculosis, listeriosis, leishmaniasis, and toxoplasmosis are caused by parasites residing the macrophages of the MPS, thus being relatively easily accessible by I.V. injected particles. The I.V. injected particles are heavily and quickly taken up by the MPS cells in case they absorb uptake promoting proteins like apolipoproteins. However, some parasites do also reside in the brain (CNS). The brain-localized parasite mostly leads to relapsing infections if not cured. Therefore, it would be of importance to target drug nanoparticles via surface modification to the brain. A successful targeting of the peptide, dalargin, to the brain using Tween 80® surface modified polyisobutylcyanoacrylates nanoparticles has been reported by Kreuter et al. [56]. A nanosuspension of Amphotericin B developed by Kayser *et al.* showed a significant improvement in its oral absorption in comparison with the

conventional commercial formulations [57]. In case of I.V administration the particle size less than 5µm is preferred. The particle size in nano range will favour the passage of the drug particles into the small capillaries in the body without any blockade. A stable intravenously injectable formulation of omeprazole has been prepared to prevent the degradation of orally administered omeprazole [37].

Aqueous suspensions of the drug can be easily nebulised and given by pulmonary route as the particle size is very less. Different types of nebulisers are available for the administration of liquid formulations. Some of the drugs successfully tried with pulmonary route are budesonide, ketotifen, ibuprofen, indomethacin, nifedipine, itraconazole, interleukin-2, p53 gene, leuprolide, doxorubicin etc. [58] Nanosuspensions can be used for targeted delivery also as the surface of the particle can be suitably modified to make them target specific. Kayser formulated a nanosuspension of Aphidicolin to improve drug targeting against leishmania-infected macrophages [26]. Scholer et al. Prapeared a nanosuspension formulation of Atovaquone and showed an improved drug targeting to the brain in the treatment of toxoplasmic encephalitis in a new murine model infected with Toxoplasma gondii [55].

CONCLUSIONS: Nanosuspensions are chiefly seen as vehicles for administering poorly water soluble drugs have been largely solved the dissolution problems to improve drug absorption and bioavailability. Nanosuspension technology can be combined with traditional dosage forms: tablets, capsules, pellets, and can be used for parenteral products. They have recently received increasing attention as colloidal carriers for targeted delivery of various anticancer drugs, photosensitizers, neutron capture therapy agents or diagnostic agents. Because of their submicron size they are easily targeted to the tumour area. Moreover the possibility of surface functionalization with a targeting moiety has open new avenues for targeted delivery of drugs, genes, photosensitizers and other molecules to the desired area. To take advantage of nanosuspension drug delivery, simple formation technologies and variety applications, nanosuspensions will continue to be of interest as oral formulations and non-oral administration develop in the future. It is expected that future research and development work will be carried out in the near future for clinical realization of these targeted delivery vehicle.

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EXHIBIT 4

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Physical and chemical stability of drug nanoparticles ☆

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ABSTRACT

As nano-sizing is becoming a more common approach for pharmaceutical product development, researchers are taking advantage of the unique inherent properties of nanoparticles for a wide variety of applications. This article reviews the physical and chemical stability of drug nanoparticles, including their mechanisms and corresponding characterization techniques. A few common strategies to overcome stability issues are also discussed.

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Contents

1. Introduction

With significant attention focused on nanoscience and nanotechnology in recent years, nanomaterial-based drug delivery has been propelled to the forefront by researchers from both academia and industry [1–3]. Various nano-structured materials were produced and applied to drug delivery such as nanoparticles [4], nanocapsules [5],

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nanotubes [6], micelles [7], microemulsions [8] and liposomes [9]. In general, the term "nanoparticles" refers to particles with sizes between 1 and 100 nm. However, submicron particles are also commonly referred as nanoparticles in the field of pharmaceutics and medicine [10–14]. Nanoparticles are categorized as nanocrystals [10], polymeric [15], liposomal [9] and solid lipid nanoparticles (SLN) [16] depending on their composition, function and morphology. Given the extensive available literature reviews on SLN, polymeric and liposomal nanoparticles [4,9,15–18], this article will focus only on nanocrystals (pure drug nanoparticles).

The unique nano-scale structure of nanoparticles provides significant increases in surface area to volume ratio which results in notably different behavior, both *in-vitro* and *in-vivo*, as compared to the traditional microparticles [10–12]. Consequently, drug nanocrystals have been extensively used in a variety of dosage forms for different purposes [10,11,14,19,20], such as improving the oral bioavailability of poorly water-soluble drugs by utilizing enhanced solubility and dissolution rate of nanoparticles [21–23]. In the field of pulmonary drug delivery, the nanoparticles are able to deliver the drugs into the deep lungs, which is of great importance for systemically absorbed drugs [11,14]. In addition, injection of poorly water-soluble nanosuspension drugs is an emerging and rapidly growing field that has drawn increasing attention due to its benefits in reducing toxicity and increasing drug efficacy through elimination of co-solvent in the formulation [10,20].

Despite the advantages of drug nanocrystals, they present various drawbacks including complex manufacturing [12,24–26], nanotoxicity [27] and stability issues [10,19,20]. Stability is one of the critical aspects in ensuring safety and efficacy of drug products. In intravenously administered nanosuspensions, for example, formation of larger particles (>5 μm) could lead to capillary blockade and embolism [20], and thus drug particle size and size distribution needs to be closely monitored during storage. The stability issues of drug nanoparticles could arise during manufacturing, storage and shipping. For instance, the high pressure or temperature produced during manufacturing can cause crystallinity change to the drug particles [12,26,28]. Storage and shipping of the drug products may also bring about a variety of stability problems such as sedimentation, agglomeration and crystal growth [29–31]. Therefore, stability issues associated with drug nanocrystals deserve significant attention during pharmaceutical product development. This article reviews existing literature on drug nanoparticle stability, including theory/mechanisms, methods used to tackle the stability problems and characterization techniques, and provides recommendations to improve the current practices. Since the stability issues related to nanoparticle dry powders are usually trivial, this review will only focus on stability of nanosuspensions (drug nanoparticles dispersed in a liquid medium).

2. Stability of drug nanoparticles

2.1. Effect of dosage form on stability

The unique characteristics of drug nanoparticles have enabled their extensive application in various dosage forms including oral, parenteral, ocular, pulmonary, dermal and other specialized delivery systems [10,11,13,20,32]. Although different dosage forms may share some common stability issues, such as sedimentation, particle agglomeration or crystal growth, their effects on drug products are quite different. For instance, particle agglomeration could be a major issue in pulmonary drug delivery since it affects deposition amount/site, and thus drug efficacy. On the other hand, agglomeration in intravenous formulations can cause blood capillary blockage and obstruct blood flow. Moreover, the selection of stabilizers is also closely related to dispersion medium, dosage form and strictly governed by FDA regulations. To date, there is a wide variety of choices on the approved stabilizers for oral dosage form whereas the excipients allowed for inhalation are very limited [33].

Drug nanoparticles exist in the final drug products either in dry powder or suspension form. Examples of the dry powder form include the dry powder inhaler, lyophililized powder for injection and oral tablets or capsules. Solid dosage forms usually have good storage stability profiles, which is why a common strategy to enhance nanosuspension stability is to transform the suspension into solid form [19,25]. Most of the reported stability concerns arise from nanosuspensions in which the drug nanoparticles are dispersed in a medium with or without stabilizers. In addition, mechanisms involved in the stability of small and large biomolecule formulations are different due to their molecular structure differences. A small molecule drug is defined as a low molecular weight non-polymeric organic compound while large biomolecules refer to large bioactive molecules such as protein/peptide. One of the major issues with protein/peptide stability is to maintain the 3-dimensional molecular conformation, such as secondary and tertiary structure in order to keep their biological activities [34,35], whereas there is no such concern for small organic molecules.

2.2. General stability issues related to nanosuspensions

Stability issues associated with nanosuspensions have been widely investigated and can be categorized as physical and chemical stability. The common physical stability issues include sedimentation/creaming, agglomeration, crystal growth and change of crystallinity state.

2.2.1. Sedimentation or creaming

Drug particles can either settle down or cream up in the formulation medium depending on their density relative to the medium. The sedimentation rate is described by Stokes' law [36,37] which indicates the important role of particle size, medium viscosity and density difference between medium and dispersed phase in determining the sedimentation rate. Decreasing particle size is the most common strategy used to reduce particle settling. Matching drug particles density with medium or increasing medium viscosity are the other widely used approaches to alleviate sedimentation problems [37,38]. Fig. 1 shows different sedimentation types that occur in suspension formulations.

In a deflocculated suspension (Fig. 1a), particles settle independently as small size entities resulting in a slow sedimentation rate. However, densely packed sediment, known as caking [39], is usually formed due to the pressure applied on each individual particle. This sediment is very difficult to be re-dispersed by agitation [36,37,39] and would be detrimental to the drug products performance. In the flocculated suspension (Fig. 1b), the agglomerated particles settle as loose aggregates instead of as individual particles [36,37]. The loose aggregates have a larger size compared to the single particle, and thus higher sedimentation rate. The loose structure of the rapidly settling flocs contains a significant amount of entrapped medium and this structure is preserved in the sediment. The final flocculation volume is therefore relatively large and the flocs can be easily broken and re-dispersed by simple agitation. K.P. Johnston et al. [40,41] have recently attempted to achieve stable nanosuspensions via a novel design of flocs structure called "open flocs", as illustrated in Fig. 1c. Thin film freezing was used to produce BSA nanorods with aspect ratio of approximately 24. These BSA nanorods were found to be highly stable when dispersed into hydrofluoroalkane (HFA) propellant, with no apparent sedimentation observed for 1 year. Due to the high aspect ratio of BSA nanorods and relatively strong attractive Van der Waals (VDW) forces at the contact sites between the particles, primary nanorods were locked together rapidly as an open structure upon addition of HFA, inhibiting collapse of the flocs [41]. The low-density open flocs structure was then filled with liquid HFA medium, preventing particle settling. Similar results were shown using needle

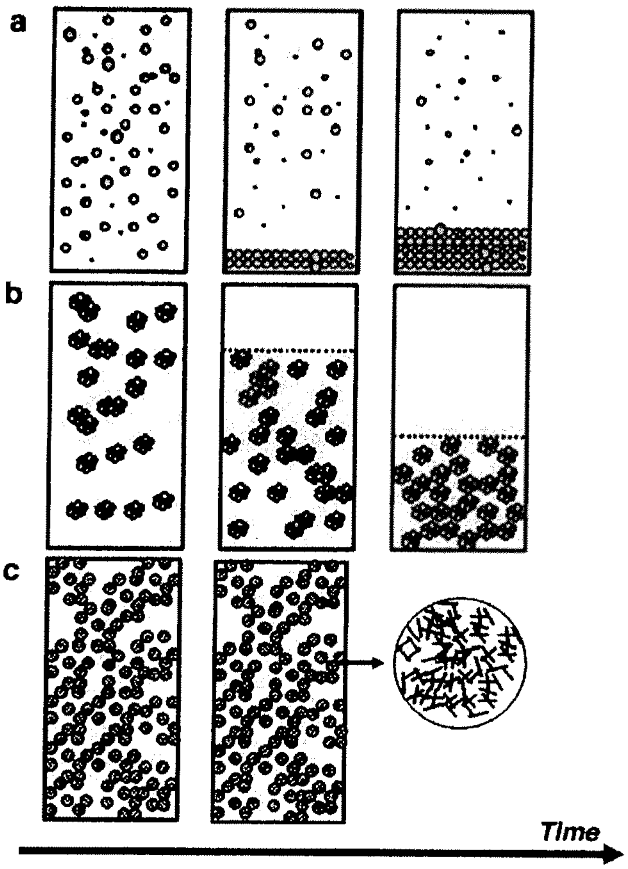

Fig. 1. Sedimentation in (a) deflocculated suspension; (b) flocculated suspension; and (c) open flocs-based suspension.

and plate shaped itraconazole nanoparticles with aspect ratios between 5 and 10 [40].

Although sedimentation is one of the key issues for colloidal suspension, the reported studies examining sedimentation issues in aqueous-based nanosuspensions are very scarce. This could be due to (i) surfactants are generally used in most of the nanosuspensions to inhibit particle agglomeration in the medium, which alleviates the sedimentation issues and (ii) the small nano-sized particles significantly reduce the sedimentation rate. In addition, many of the aqueous nanosuspensions are transformed to dry solid form by spray drying or freeze drying to circumvent the long-term sedimentation issue. Unfortunately, this solidification process cannot be applied to non-aqueous nanosuspensions where sedimentation/creaming is commonly present. An example to illustrate this is metered dose inhaler (MDI) formulations where the nanoparticles are suspended in HFA propellants. Sedimentation or creaming is a key aspect affecting stability of these formulations. Particle engineering to optimize particle surface properties and morphology, e.g. hollow porous particles [42], and introduction of surfactant(s) is generally employed to alleviate the issue.

2.2.2. Agglomeration

The large surface area of nanoparticles creates high total surface energy, which is thermodynamically unfavorable. Accordingly, the particles tend to agglomerate to minimize the surface energy. Agglomeration can cause a variety of issues for nanosuspensions including rapid settling/creaming, crystal growth and inconsistent dosing. The most common strategy to tackle this issue is to introduce stabilizers to the formulation. In addition to safety and regulation considerations, selection of stabilizers is based on their ability to provide wetting to surface of the particles and offer a barrier to prevent nanoparticles from agglomeration [13,19].

There are two main mechanisms through which colloidal suspensions can be stabilized in both aqueous and non-aqueous medium, i.e. electrostatic repulsion and steric stabilization [10,36,37]. These two mechanisms can be achieved by adding ionic and non-ionic stabilizers into the medium, respectively. Stabilization from electrostatic repulsion can be described by the classic Derjaguin–Landau–Verwey–Overbeek (DLVO) theory [43,44]. This theory mainly applies to aqueous suspension while its application in non-aqueous medium is still not well-understood [33]. The DLVO theory assumes that the forces acting on the colloidal particles in a medium include repulsive electrostatic forces and attractive VDW forces. The repulsive forces are originated from the overlapping of electrical double layer (EDL) surrounding the particles in the medium, and thus preventing colloidal agglomeration. The EDL consist of two layers: (i) stern layer composed of counter ion attracted toward the particle surface to maintain electrical neutrality of the system and (ii) Gouy layer which is essentially a diffusion layer of ions (Fig. 2).

The total potential energy (V_T) of particle–particle interaction is a sum of repulsion potential (V_R) generated from electric double layers and attraction potential (V_A) from the VDW forces. V_A is determined by the Hamaker constant, particle size and inter-particulate distance while V_R depends on particle size, distance between the particles, zeta potential, ion concentration and dielectric constant of the medium. V_R is extremely sensitive to ion concentration in the medium. As the ion strength is increased in the medium, the thickness of EDL decreases due to screening of the surface charge [36,37]. This causes decrease in V_R, increasing the susceptibility of the dispersed particles to form aggregates. Zeta potential (ZP) is electric potential at the shear plane which is the boundary of the surrounding liquid layer attached to the moving particles in the medium. ZP is a key parameter widely used to predict suspension stability. The higher the ZP, the more stable the suspension is.

In the case of steric stabilization, amphiphilic non-ionic stabilizers are usually utilized to provide steric stabilization which is dominated by solvation effect. As the non-ionic stabilizers are introduced into nanosuspensions, they are absorbed onto the drug particles through an anchor segment that strongly interacts with the dispersed particles, while the other well-solvated tail segment extends into the bulk medium (Fig. 3).

As two colloidal particles approach each other, the stabilizing segments may interpenetrate, squeezing the bulk medium molecules out of the inter-particulate space as illustrated in Fig. 3. This interpenetration is thermodynamically disfavored when a good solvent is used as the bulk medium to stabilize the tail [36]. Accordingly, provided that the stabilizers can be absorbed onto the particle surface through the anchor segment, strong enthalpic interaction (good solvation) between the solvent and the stabilizing segment of the stabilizer is the key factor to achieve steric stabilization and prevent particles from agglomeration in the medium [36,37]. In addition to solvation, the stabilizing moiety needs to be sufficiently long and dense to maintain a steric barrier that is capable of minimizing particle–particle interaction to a level that the VDW attractive forces are less than the repulsive steric forces [43–45].

The main drawback associated with the steric stabilization is the constant need to tailor the anchoring tail according to the particular drug of interest. Due to the lack of fundamental understanding of interaction between the stabilizers and dispersed nanoparticles, current surfactant screening approaches to achieve a successful steric stabilization are mostly empirical and could be very burdensome [45–49]. In addition, the solvation of the stabilizing segment is susceptible to variation in temperature. Stabilizer concentration could also play a role in causing suspension instability by affecting the absorption affinity of non-ionic stabilizers to drug particles surface. Deng et al.

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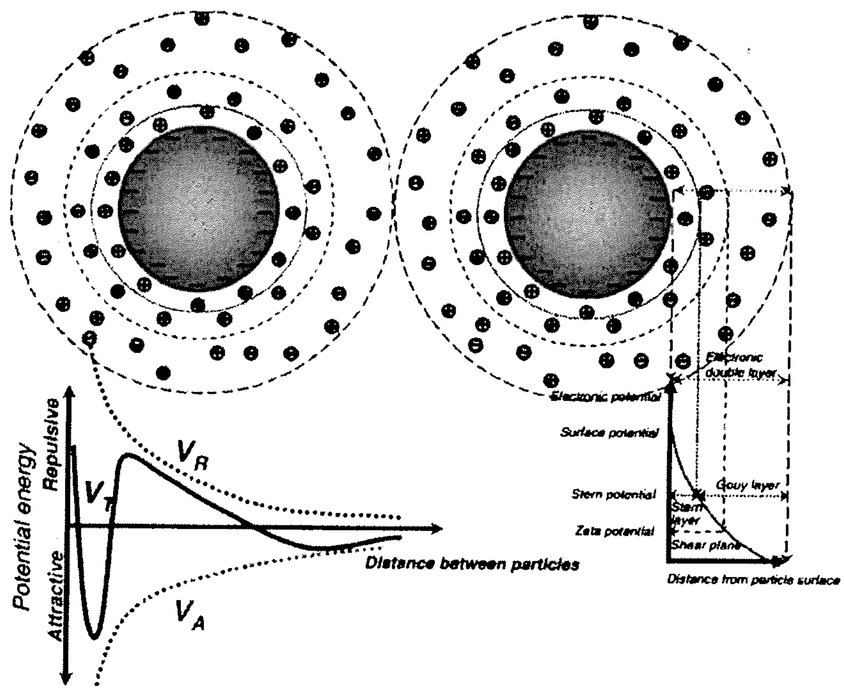

Fig. 2. Illustration of classical DLVO theory. Attractive forces are dominant at very small and large distances, leading to primary and secondary minimum, while repulsive forces are prevailing at intermediate distances and create net repulsion between the dispersed particles, thus preventing particle agglomeration.

[50] used Pluronic® F127 to stabilize paclitaxel nanosuspensions. It was reported that stabilizers had high affinity to nanocrystals surface at concentrations below critical micelle concentration (CMC), and increasing concentrations above CMC caused loss of F127 affinity to the nanocrystals and thus unstable formulation. This was because F127 monomers on the nanocrystals surface started to aggregate with each other to form micelles as the concentration was increased to the CMC level, leading to a lower affinity to the drug crystals. Temperature was also shown to affect the stabilizer affinity to drug crystals. This is expected since the CMC level is dependent on temperature.

It is apparent that combination of the two stabilization mechanisms can be very beneficial in achieving a stable colloidal dispersion. In addition, the combination of a non-ionic stabilizer with an ionic stabilizer reduces the self repulsion between the ionic surfactant molecules, leading to closer packing of the stabilizer molecules [10,51].

Besides the steric and electrostatic stabilization mechanisms, some other stabilization mechanisms have also been reported. Makhlof et al. produced indomethacin (IMC) nanocrystals using the emulsion solvent diffusion technique [52]. The nanoparticles were stabilized using various cyclodextrins (CyDs) without adding any surfactants. The stabilizing effect was attributed to the formation of a CyD network in the aqueous medium via intermolecular interaction of CyD molecules. The network-like structure was believed to prevent aggregation and crystal growth of IMC nanoparticles initially produced from the solvent diffusion process. Similar stabilization mechanism was also observed in another study where budesonide microsuspension was stabilized with hydroxypropyl-beta-cyclodextrin in HFA medium [53]. Another approach to enhance suspension stability that has increasingly been utilized is engineering of particle morphology. One breakthrough in this area was the porous particle

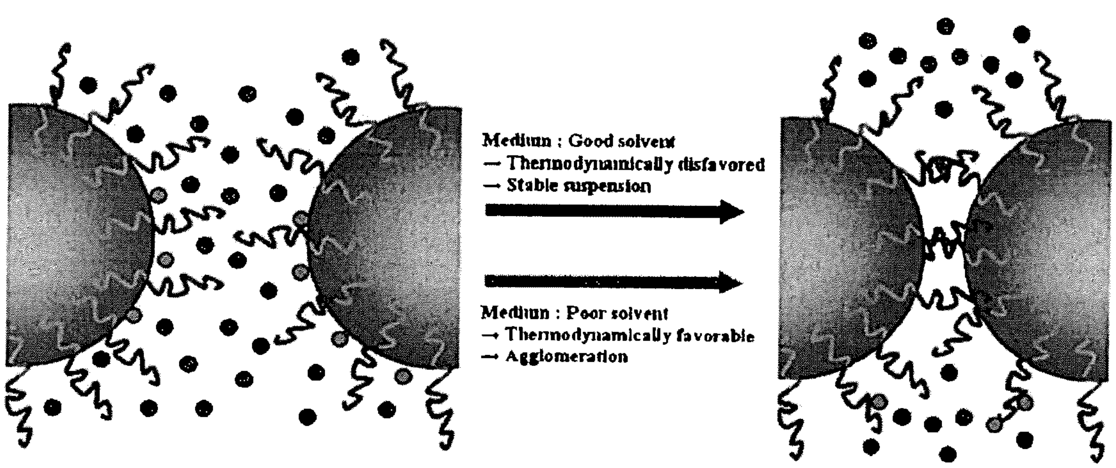

Fig. 3. Steric stabilization mechanisms according to Gibbs free energy: $\Delta G = \Delta H - T\Delta S$. A positive ΔG indicates stable suspension while negative ΔG induces particle agglomeration. If the medium is a good solvent for the stabilizing moiety, the adsorbed stabilizing layers on the dispersed particles cannot interpenetrate each other when the particles collide. This reduces the number of configurations available to the adsorbed stabilizing tails, resulting in a negative entropy change and positive ΔG. On the other hand, if the dispersion medium is a poor solvent, the adsorbed layers on the particles may interpenetrate thermodynamically and induces particles agglomeration.

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concept that was first introduced by Edwards et al. [54]. The porous particles include hollow porous particle [42] and porous nanoparticle-aggregate particles (PNAPs) [14]. Unfortunately, most of the work has been focused on microsuspension or polymeric colloidal formulations and has not been applied to pure drug nanoparticles.

Table 1 summarizes a few published studies on pharmaceutical nanosuspensions. Due to the vast amount of literature work on the pharmaceutical nanosuspensions, this review will focus only on the studies that provide a more profound enlightenment on the stabilizer selection for nanosuspensions. The summary table shows that most of nanosuspensions were generated in aqueous medium, with only a limited number of nanosuspensions made in non-aqueous environment. The commonly used ionic stabilizers in aqueous medium include sodium dodecyl sulfate (SDS), sodium lauryl sulfate (SLS), lecithin and docusate sodium. The non-ionic surfactants used in aqueous medium are usually selected from Pluronic® surfactants, Tween 80, polyethylene glycol (PEG), polyvinyl alcohol (PVA) polyvinylpyrrolidone (PVP) and cellulose polymers such as hydroxypropyl cellulose (HPC) and hydroxypropyl methylcellulose (HPMC).

The stabilizers are not only used to provide short- and long-term storage stability for nanosuspensions, but also to achieve successful formation and stabilization of nanocrystals during particle production. Lee et al. designed and synthesized various amino acid copolymers containing lysine as the hydrophilic segments with alanine, phenylalanine or leucine as hydrophobic moieties [49]. Wet comminution was used to produce naproxen nanosuspensions in presence of HPC and amino acid copolymers. Lysine copolymer with alanine was unable to produce submicron particles while the other copolymers with phenylalanine and leucine were capable of forming the nanoparticles. The size of nanocrystals was proven to be constant over 1 month storage and the crystallinity was also shown to be preserved after the wet comminution process. Furthermore, hydrophobicity of the copolymers was identified as the key factor in achieving the stable nanosuspensions, attributed to strong polymer adsorption onto the hydrophobic drug surfaces. Although this work did not provide an in-depth discussion on how the copolymers interacted with the drug nanoparticles, it illustrated the importance of careful selection of the anchor group (that is attached to the drug surface) in facilitating the production of a stable nanosuspesion. In the subsequent study [45], they attempted to understand the nature of interactions between polymeric stabilizers and drugs with different surface energies. Nanocrystals of seven model drugs with PVP K30 and HPC as stabilizers were generated using wet comminution. It was expected that a close match of surface energy between the stabilizers and drug crystals would promote the absorption of stabilizers onto drug particles, and thus help in reducing the particle size during the wet comminution process. Although surface energy did not seem to correlate well with particle size for HPC stabilized system, some trend was observed for PVP stabilized suspension with only one exception.

A further study with seven stabilizers (non-ionic stabilizers: HPC, PVP K30, Pluronic® F127 & F68, PEG and ionic stabilizers: SDS and benzethoinum chloride) and eleven model drugs was conducted by the same group in order to provide more understanding on the stabilization mechanism [48]. Again, the general trend between surface energy and particle size reduction was not observed in this work. PEG was unsuccessful in reducing the particle size of most drug candidates while the other non-ionic stabilizers proved to be effective in reducing the size of five drug candidates that had similar surface energies to the stabilizers. F68 was shown to be the most effective stabilizer (successfully stabilizing nine drug candidates), which could be due to its strong chain adsorption onto the drug crystals through the hydrophobic polypropylene glycol (PPG) units. F127 was found to be less efficient than F68 likely because the short processing time led to inefficient physical adsorption of higher molecular weight F127 to the drug surface. This study demonstrated that a combination of ionic and non-ionic stabilizers is not always beneficial to enhance stabilization. A few combinations of SDS or benzethoinum chloride with various non-ionic stabilizers resulted in positive stability effects while the others did not. The effects of physicochemical properties of the drugs on the stabilization were also explored in this study. In general, drugs with lower aqueous solubility, higher molecular weight and higher melting point were shown to have higher chance for successful nanosuspension formation.

Van Eerdenbrugh et al. conducted an expanded study using 13 stabilizers at 3 different concentrations to stabilize 9 drug compounds [47]. The particles were generated using the wet milling technique. The success rate in producing nanosuspensions using polysaccharide based stabilizers [HPMC, methylcellulose (MC), hydroxyethylcellulose (HEC), HPC, carboxymethylcellulose sodium (NaCMC), alginic acid sodium (NaAlg)] was limited by the high viscosity of these polymeric stabilizer solutions. Increasing concentration of these stabilizers did not appear to be helpful. In contrast, the other stabilizers [PVP K30, PVP K90, PVA, Pluronic® F68, polyvinyl alcohol–polyethylene glycol graft copolymer (K-IR), Tween 80 and D-α-tocopherol polyethylene glycol 1000 succinate (TPGS)] did not encounter the viscosity issue. PVA was ineffective in producing the nanosuspension and the success probability of PVP K30, PVP K90, F68 and K-IR is highly dependent on their concentration. Higher concentrations (25 wt.% and 100 wt.%) increased the stabilizing efficacy significantly. Tween 80 and TGPS were proven to be most effective stabilizers. Addition of TGPS (at concentrations >25 wt.%) allowed nanosuspension formation for all tested drug compounds. No correlation was observed between drug physicochemical properties (molecular weight, melting point, log p, solubility and density) and nanosuspension formation success rate. It was demonstrated that surface hydrophobicity of the drug candidates was the driving force for nanoparticles agglomeration, thus lowering the success rate of nanosuspension production.

Mishra et al. explored nanosuspension stability issues during both production and storage [29]. Hesperetin nanosuspensions were produced using HPH with Pluronic® F68, alkyl polyglycoside (Plantacare 2000) and inulin lauryl carbamate (Inutec SP1), or Tween 80 as stabilizers. It was demonstrated that all stabilizers were suitable for successful production of hesperetin nanosuspensions. The size of nanocrystals was dependent on power density applied in the homogenization process and the hardness of the crystals. The effect of stabilizers on the particle size was negligible. Short-term stability over a period of 30 days was examined in order to evaluate the stabilizer efficiency. ZP was measured as a key parameter to predict the stability. In distilled water, the ZP values of all the nanosuspensions fell between −30 and −50 mV and the values dropped significantly in the original dispersion medium. This can be explained by the fact that adsorbed layers of large molecules shifted the shear plane to a longer distance from the particle surface, thus reducing the measured value of zeta potential (Fig. 4). However, the low ZP value does not point to an unstable suspension in this case, which could be due to the additional presence of steric stabilization mechanism. Both Inutec and Plantacare stabilized nanosuspensions also showed significant reduction of ZP measured from water to dispersion medium, indicating a thick absorbed steric layer and good stability. F68 exhibited only slight decrease in ZP, indicating a relatively thin stabilization layer. The ZP value of Tween 80 was only −13 mv in the dispersion medium, pointing to a potentially problematic stabilization. The study demonstrated that zeta potential measurement is a good predictor for storage stability. Nanosuspensions stabilized by Inutec and Plantacare were stable at all storage conditions (4, 25 and 40 °C) up to 30 days while F68 stabilized nanosuspensions were shown to be less stable. The Tween 80 formulation stability was the poorest. Pardeike et al. [30] conducted a similar study using phospholipase A2 inhibitor PX-18 nanosuspensions produced by HPH with Tween 80 as stabilizer. In this work, ZP of the homogenized nanosuspensions was dropped from −50 mV to

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t1.1 **Table 1**

t1.2 Literature summary of pharmaceutical nanosuspensions.

	Nanoparticles compound	Manufacturing technique	Delivery route	Dispersion medium	Stabilizers	Reference
t1.3						
t1.4	Oridonin	HPH	NA	Water	PVP K25, Brij 78, SDS, Pluronic® F68, lecithin	Gao et al. (2007) [55]
t1.5	Oridonin	HPH	IV	Water	Pluronic® F68, lecithin	Gao et al. (2008) [56]
t1.6	Budesonide	HPH	Inhalation	Water	Lecithin, Span 85, tyloxapol, cetyl alcohol	Jacobs et al. (2002) [57]
t1.7	Buparvaquone	HPH	Inhalation	Water	Pluronic® F68 and PVA	Hernadez-Trejo et al.(2005) [58]
t1.8	Buparvaquone	HPH	Oral	Water	Pluronic® F68 and lecithin	Jacobs et al. (2002) [59]
t1.9	Diclofenac acid	HPH	Oral	Water	Pluronic® F68	Lai et al. (2009) [60]
t1.10	Azothromycin	HPH	NA	Water	Lecithin, Pluronic® F68, Tween 80	Zhang et al. (2007) [61]
t1.11	Rutin	HPH	Oral	Water	SDS	Mauludin et al. (2009) [62]
t1.12	Rutin	HPH	Oral	Water	SDS, Tween 80, Pluronic® F68, PVA	Mauludin et al. (2009) [63]
t1.13	Tarazepide	HPH	NA	Water	Tween 80, Pluronic® F68	Jacobs et al. (2000) [64]
t1.14	Omeprazole	HPH	IV	Water	Pluronic® F68	Moschwitzer, (2004) [65]
t1.15	Amphotericin B	HPH	Oral	Water	Tween 80, Pluronic® F68	Kayser et al. (2003) [22]
t1.16	Nimodipine	HPH	IV	Water	Pluronic® F68, sodium cholic acid and mannitol	Xiong et al., (2008) [66]
t1.17	Albendazole	HPH	Oral	Water	SLS, Carbopol, PS 80, hpmc	Kumar et al. (2008) [23]
t1.18	RMKP 22	HPH	NA	Water	Phospholipon 90	Peters et al. (1999) [67]
t1.19	Hesperetin	HPH	Dermal	Water	Pluronic® F68, Inutec SP1, Tween 80 and Plantacare 2000	Mishra et al. (2009) [29]
t1.20	Hydrocortisone, prednisolone and dexamethasone	HPH	Opthalmic	Water	Pluronic® F68	Kassem et al. (2007) [68]
t1.21	Ascorbyl palmitate	HPH	NA	Water	SDS, Tween 80	Teeranachaideekul et (2008) [69]
t1.22	RMKK99	HPH	NA	Water	Potassium oleate, Tween 80	Krause et al. (2001) [70]
t1.23	Nifedipine	HPH	NA	Water	HPMC	Hecq et al. (2005) [71]
t1.24	Undisclosed	HPH	Oral	Water	SLS, HPMC, PVA, Acaciae Gum, Pluronic® F127	Hecq et al. (2006) [72]
t1.25	Hydroxycamptothecin	HPH	NA	Water	Lipoid S75, Pluronic® F68, Solutol® HS 15	Zhao et al. (2010) [73]
t1.26	Asulacrine	HPH	IV	Water	Pluronic® F68	Ganta et al. (2009) [74]
t1.27	RMKP 22	HPH	NA	Water	Tween 80	Muller et al. (1998) [75]
t1.28	RMKP 22	HPH	NA	Water	Tween 80, Glycerol	Grau et al. (2000) [76]
t1.29	PX-18	HPH	NA	Water	Tween 80	Pardeike et al. (2010) [30]
t1.30	PX-18	HPH	NA	Water	Tween 80	Wang et al. (2010) [77]
t1.31	Silybin	HPH	Oral, IV	Water	Lecithin, Poloxamer 188	Wang et al. (2010) [78]
t1.32	Tarazepide	HPH	IV	Water	Pluronic® F68, Tween 80, Glycerol	Jacobs et al. (2000) [64]
t1.33	Omeprazole, albendazole and danazol	Wet milling	Oral	Water	Pluronic® F108, F68	Tanaka et al. (2009) [79]
t1.34	Fluticasone, budesonide	Wet milling	Inhalation	Water	Tween 80	Yang et al. (2008) [80]
t1.35	Naproxen	Wet milling	NA	Water	HPC, arginie hydrochloride	Ain-Ai et al. (2008) [81]
t1.36	Loviride	Wet milling	NA	Water	Tween 80, Pluronic® F68	Van Eerdenbrugh et al. (2007) [82]
t1.37	Nine different compounds	Wet milling	NA	Water	13 different stabilizers	Van Eerdenbrugh et al. (2009) [47]
t1.38	Zinc Insulin	Wet milling	NA	Water	Pluronic® F68, sodium deoxycholate	Merisko-Liversidge et al. (2004) [83]
t1.39	Ethyl Diatrizoate	Wet milling	NA	Water	Poloxamine 908	Na et al. (1999) [84]
t1.40	Cinnarizine, itraconazole and phenylbutazone	Wet milling	NA	Water	TPGS 1000	Van Eerdenbrugh et al. (2008) [85]
t1.41	Nine different compounds	Wet milling	NA	Water	TPGS 1000	Van Eerdenbrugh et al. (2008) [86]
t1.42	Beclomethasone dipropionate	Wet milling	Inhalation	Water	PVA	Wiedmann et al. (1997) [87]
t1.43	Rilpivirine	Wet milling	Parenteral	Water	Pluronic® F108, TPGS 1000	Baert et al. (2009) [88]
t1.44	Undisclosed	Wet milling	NA	Water	Plasdone S-630, docusate sodium	Deng et al. (2008) [89]
t1.45	Piposulfan, etoposide, camptothecin, paclitaxel	Wet milling	NA	Water	Tween 80, Span 80, Pluronic® F108, F127	Merisko-Liversidge et al. (1996) [90]
t1.46	Naproxen	Wet comminution	NA	Water	Copolymers of amino acids	Lee et al. (2005) [49]
t1.47	Seven different compounds	Wet comminution	NA	Water	HPC, PVP	Choi et al. (2005) [45]
t1.48	Eleven different compounds	Wet comminution	NA	Water	HPC, PVP, PEG, SDS, Pluronic® F68, F127, benzethonium chloride	Lee et al. (2008) [48]
t1.49	Dihydroartemisinin	Vibrational rod milling	NA	Water	PVP K30, sodium deoxycholate	Chingunpitak et al.(2008) [91]
t1.50	Probucol	Vibrational rod milling	NA	Water	PVP, SDS	Pongpeerapat et al. (2008) [92]
t1.51	Ibuprofen	Precipitation, microfluidization	NA	Water	SLS, PVP K30, Pluronic® F68, F127, Tween 80, HPMC	Verma et al. (2009) [31]
t1.52	Hydrocortisone	Precipitation, microfluidization	NA	Water	PVP, HPMC, SLS	Ali et al. (2009) [93]
t1.53	Ibuprofen	Solvent diffusion, melt emulsification	NA	Water	PVA, PVP K25, Pluronic® F68, Tween 80,	Kocbek et al. (2006) [94]
t1.54	Alendronate-gallium, alendronate-gadolinium	Complex precipitation	NA	Water	None	Epstein et al. (2007) [95]
t1.55	Paclitaxel	Stabilization of nanocrystal (SNC)	NA	Water	Pluronic® F127	Deng et al. (2010) [50]
t1.56	Felodipine	Antisolvent precipitation & Wet-milling	NA	Water	PVP K30, SDS, docusate sodium	Lindfors (2007) [96]
t1.57	Naproxen	Antisolvent precipitation	Oral	Water	PVP K15, Pluronic® F127	Chen et al. (2009) [97]
t1.58	Carbamazepine	Antisolvent precipitation	NA	Water	HPMC, PVP K17	Douroumis et al. (2007) [98]
t1.59	Cyclosporin A	Antisolvent precipitation	Inhalation	Water	Tween 80	Tam et al. (2008) [99]
t1.60	Undisclosed	Antisolvent precipitation, Wet milling	IV, Oral	Water	PVP, SDS, Miglyol, docusate sodium	Sigfridsson et al. (2007) [100]
t1.61	β-methasone valerate-17, oxcarbazepine	Antisolvent precipitation	NA	Water	HPMC, lipoid S75, PEG-5 soy sterol	Douroumis et al. (2006) [101]
t1.62	Retinoic acid	Antisolvent precipitation	NA	Water	None	Zhang et al. (2006) [102]

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t1.63 **Table 1** (*continued*)

	Nanoparticles compound	Manufacturing technique	Delivery route	Dispersion medium	Stabilizers	Reference
t1.65	2-devinyl-2-(1-hexyloxyethyl) pyropheophorbide	Antisolvent precipitation	NA	Water	None	Baba et al. (2007) [103]
t1.66	Nitrendipine	Precipitation–ultrasonication	Oral	Water	PVA	Xia et al. (2010) [104]
t1.67	Indomethancin	Emulsion diffusion	NA	Water	Cyclodextrins	Makhlof et al. (2008) [52]
t1.68	Celecoxib	Emulsion diffusion	Oral	Water	Tween 80, PVP K30, SDS	Dolenc et al. (2009) [105]
t1.69	Griseofulvin	Emulsion diffusion	NA	Water	Tween 80, Oramix CG-110	Trotta el al.(2003) [106]
t1.70	Mitotane	Emulsion diffusion	NA	Water	Tween 80, caprylyl-capryl glucoside, lecithin	Trotta el al.(2001) [107]
t1.71	Griseofulvin	Microemulsion diffusion	NA	Water	Lecithin	Trotta el al.(2003) [108]
t1.72	Lysozyme	Emulsification/freeze-drying	Inhalation	HFA	None	Nyambura et al.(2009) [109]
t1.73	Bovine serum albumin	Thin film freezing	Inhalation	HFA	None	Engstrom et al.(2009) [41]
t1.74	Itraconazole	Thin film freezing	Inhalation	HFA	None	Tam et al.(2010) [40]
t1.75	Insulin	Emulsification + freeze-drying	Inhalation	HFA	Citral, cineole	Nyambura et al.(2009) [110]
t1.76	Salbutamol sulfate	Microemulsion + freeze-drying	Inhalation	HFA	Lecithin, docusate sodium	Dickinson et al. (2001) [111]
t1.77	Salbutamol sulfate	HPH	NA	Acetonitrile	Tween 80	Ahmad et al. (2009) [112]
t1.78	Horseradish peroxidase, carbonic anhydrase, lysozyme, subtilisin carlsberg and α-chymotrypsin	Freeze-drying	NA	Ethyl acetate	Methyl-β-cyclodextrin	Montalvo et al. (2008) [113]
t1.79	Diclofenac	Emulsification + freeze drying	Transdermal	Isopropyl myristate	Sucrose ester	Piao et al. (2007) [114]

around −20 mV when tested from water to dispersion medium. It is generally believed that ZP of ±20 mV is sufficient to maintain a stable formulation with a combined electrostatic and steric stabilization [30]. The PX-18 nanosuspension was shown to be physically stable (no changes in particle size distribution) for more than half year at the storage condition of 5 and 25 °C. However, physical instability was observed after 1 month storage at a higher storage temperature. This could be due to the decreased dynamic viscosity and enhanced diffusion constant at higher temperature.

There is another interesting work by Pongpeerapat et al. investigating probucol/PVP/SDS ternary ground mixture (GM) that was prepared with a vibrational rod mill [92]. The produced primary probutcol nanoparticles were around 20 nm in presence of both SDS and PVP. An interesting phenomenon was observed following the dispersion of the GM into water. For GM stabilized with PVP K17 and SDS, spherical agglomerates of primary nanocrystals were formed immediately in the size of around 90 nm after dispersion of the GM into water. A further agglomeration to around 160 nm in size occurred gradually during the storage stability study. In the case of PVP K12 and SDS, agglomerations of approximately 180 nm were observed after 4 days of storage and then remained stable up to 84 days. This phenomenon is illustrated in Fig. 5. Above critical aggregation concentration, SDS complexes with PVP to form a "necklace" structure in aqueous medium through both electrostatic and hydrophobic interactions. Following dispersion of probucol/PVP K17/SDS into water, PVP K17/SDS "necklace" complex interacted with primary drug nanoparticles, causing immediate agglomeration of the primary nanoparticles into 90 nm aggregates. The 160 nm secondary nanoparticles were formed due to further gradual agglomeration process. The stabilization of probucol nanocrystals was attributed to formation of PVP K17/SDS layered structure on the surface of probucol. For the GM of probucol/PVP K12/SDS, agglomeration of primary drug nanoparticles occurred more rapidly because of the insufficient surface coverage of PVP K12 and SDS on the probucol surface. Stabilization of the nanosuspension was linked to absorption of PVP K12 on the surface of probucol nanocrystals, owing to the absence of layered structure.

Despite the proven importance of stabilizers in preventing particle agglomeration, there have been a few studies that generated stable nanosuspensions without stabilizers. Baba et al. prepared 2-devinyl-2-(1-hexyloxyethyl)pyropheophorbide (HPPH) nanosuspensions without any stabilizer and reported formulation stability for more than 3 months [103]. The self-stabilization of the nanosuspensions was attributed to a high ZP value (−40 mv) resulting from the deprotonation of the carboxylic end group of HPPH molecules. A similar self-stabilized nanosuspension was reported in another study in which amorphous all-trans retinoic acid nanoparticles were shown to be stable in aqueous medium up to 6 months. Epstein et al. [95] prepared self-suspended alendronate nanosuspensions by combining the negative charged alendromnic acid with gallium (Ga) or

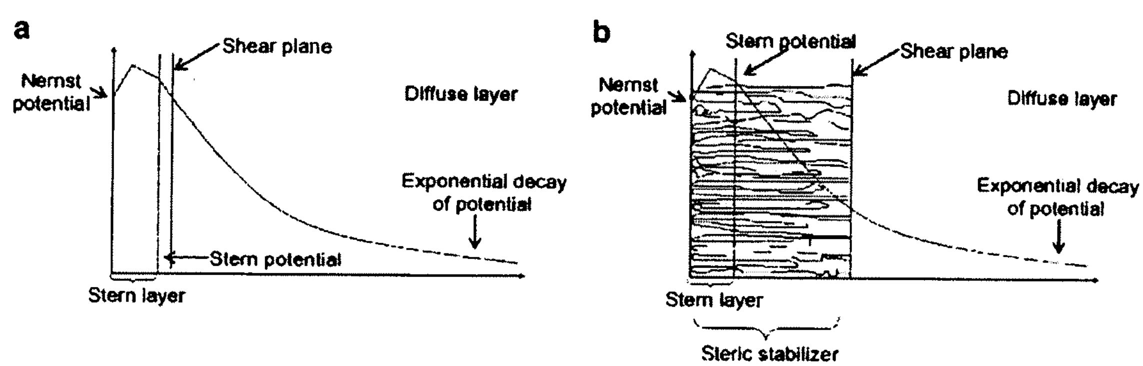

Fig. 4. Location of shear plane in an electrostatic stabilized system (a) and in a combined steric-electrostatic stabilized system (b). Reprinted from Ref. [30] with permission from ELSEVIER.

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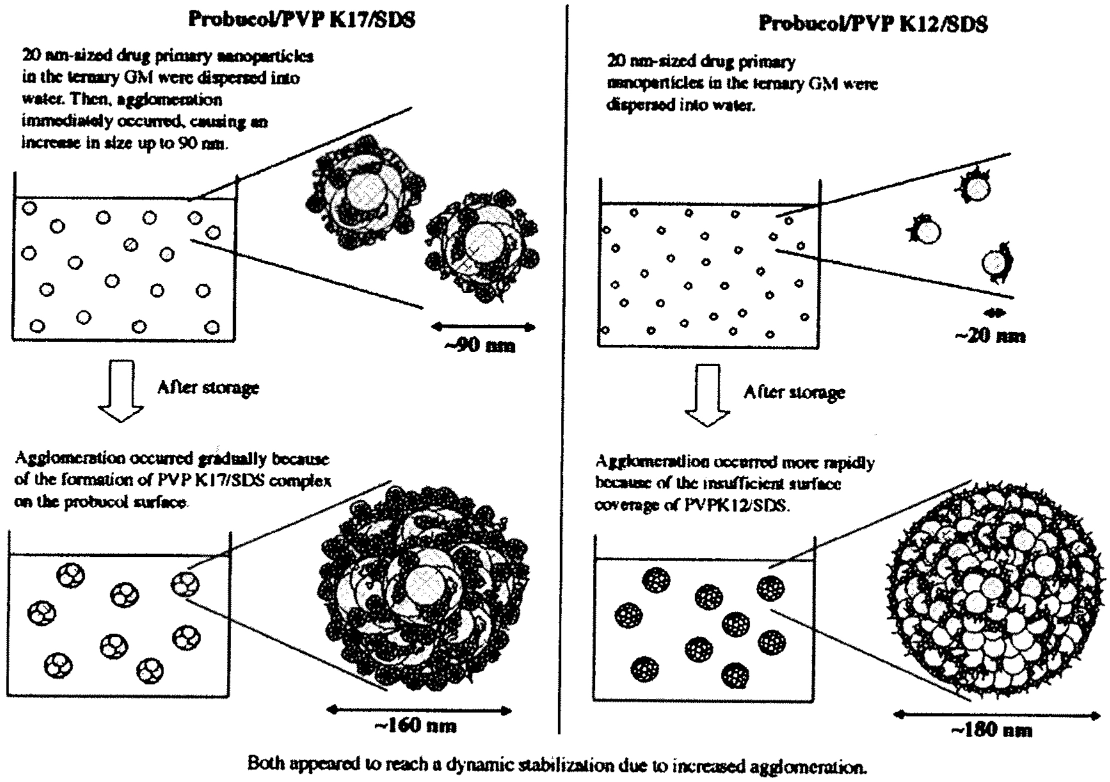

Fig. 5. Schematic overview of agglomeration/stabilization mechanism of probucol/PVP/SDS ternary ground mixture after dispersion into water. Reprinted from Ref. [92] with permission from ELSEVIER.

gadolinium (Gd) under sonication as complex nanoparticles. The alendronate-Ga nanosuspension was shown to be stable for more than 3 months, while the alendronate-Gd nanosuspension was stable for only 3 days. These stability profiles correlated well with their ZP values (33 mV for Ga complex vs. 21 mV for Gd complex).

2.2.3. *Crystal growth*

Crystal growth in colloidal suspensions is generally known as Ostwald ripening and is responsible for changes in particle size and size distribution. Oswald ripening is originated from particles solubility dependence on their size. Small particles have higher saturation solubility than larger ones according to Ostwald–Freundlich equation [115], creating a drug concentration gradient between the small and large particles. As a consequence, molecules diffuse from the higher concentration surrounding small particles to areas around larger particles with lower drug concentration. This generates supersaturated solution around the large particles, leading to drug crystallization onto the large particles. This diffusion process leaves an unsaturated solution surrounding the small particles, causing dissolution of the drug molecules from the small particles into the bulk medium. This diffusion process continues until all the small particles are dissolved. The Ostwald ripening is essentially a process where large particles grow at the expense of smaller particles [36,37], which subsequently leads to a shift in the particle size and size distribution of the colloidal suspension to a higher range. The diffusion and crystal growth during Ostwald ripening is shown schematically in Fig. 6.

A narrow particle size distribution can minimize the saturation solubility difference and drug concentration gradients within the medium, and thus help to inhibit occurrence of the Ostwald ripening [37]. This can perhaps explain why Ostwald ripening is not a major concern for nanosuspensions with uniform particle size [10,20]. Stabilizers may also alleviate Ostwald ripening as long as they do not enhance the drug solubility [116,117]. Being absorbed on the nanoparticles surface, the stabilizers can reduce the interfacial tension between the solid particles and liquid medium, and thus preventing the Ostwald ripening. Solubility, temperature, and mechanical agitation also affect Ostwald ripening [117]. Verma et al. produced ibuprofen nanosuspensions by microfluidization milling with the aid of various stabilizers (HPMC, Pluronic® F68 & F127, Kollidon 30, SLS) [31]. The particle size obtained with microfluidization showed some correlation with the ibuprofen solubility in aqueous stabilizer solutions. A higher solubility of ibuprofen in the solution of SLS, Tween 80 and Pluronic® F127 resulted in larger particles due to Ostwald ripening that occurred during process. A similar correlation was observed for ibuprofen particles during storage where Ostwald ripening was also believed to the driving factor for formation of larger particles. Van Eerdenbrugh et al. demonstrated that Ostwald ripening was highly dependent on temperature by exploring TPGS stabilized nanosuspensions for 9 different drug candidates [86]. Following 3 months storage at room temperature, Ostwald ripening occurred in 8 out of 9 nanosuspensions studied. Enhanced Ostwald ripening was observed at 40 °C storage, while lowering temperature to 4 °C slowed down or even stopped Ostwald ripening effects.

2.2.4. *Change of crystalline state*

Crystalline state is one of the most important parameters affecting drug stability, solubility, dissolution and efficacy. The main issue with crystalline state change is the transformation between amorphous and crystalline state. The high energy top-down manufacturing techniques tend to create partially amorphous nanosuspensions and some bottom-up techniques can create completely amorphous particles. The high energy amorphous particles are unstable and inclined to convert to low energy crystalline state over time. This conversion occurs depending on different parameters, such as temperature, dispersion medium, stabilizers and the presence of crystalline particles. Lindfors et al. produced Felodipine amorphous

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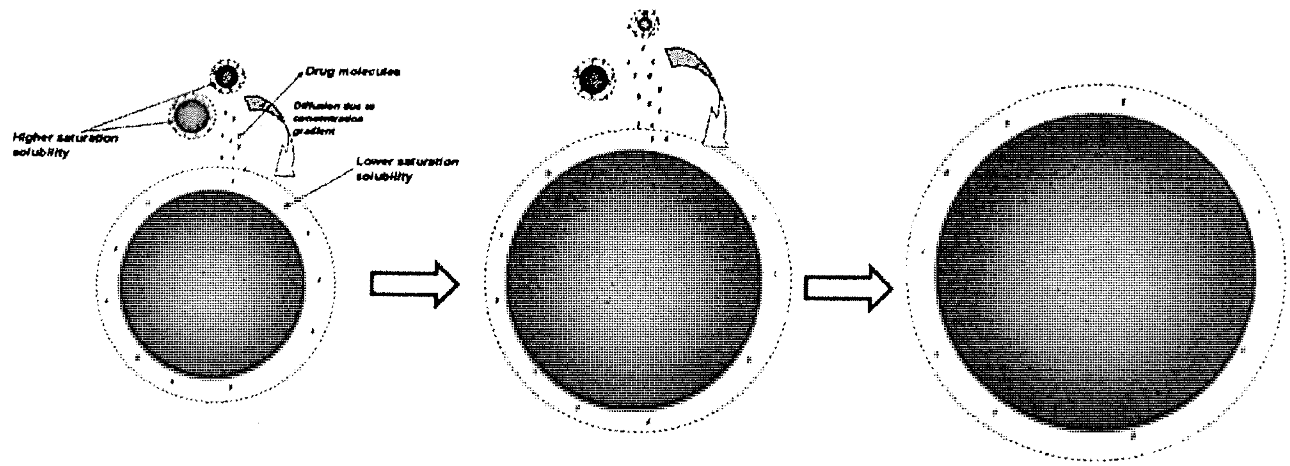


Fig. 6. Schematic illustration of Ostwald ripening.

nanosuspensions via anti-solvent precipitation under sonication [96]. They demonstrated that amorphous nanoparticles were highly unstable in the presence of small amounts of crystalline particles. This was attributed to saturation solubility differences between amorphous and crystalline nanoparticles that initiated a similar diffusion process to Ostwald ripening, leading to a rapid conversion of amorphous nanoparticles to crystalline state. Although most of amorphous particles have been shown to be unstable, a few amorphous nanosuspensions have been demonstrated to be stable over a certain period of time. Amorphous hydrocortisone nanosuspensions, produced through a bottom-up nanoprecipitation technique using microfluidic reactors, was found to remain stable after 3 months storage at room temperature [93]. Amorphous all-trans retinoic acid nanosuspensions, prepared by an anti-solvent precipitation technique, were also shown to be stable over 6 months storage at 4 °C [102].

Manufacturing process might also induce some other type of crystalline transformation. Lai et al. prepared the diclofenac acid (DCF) nanosuspensions by HPH with two different crystalline forms (DCF1 and DCF2) [60]. 5 w/w% Pluronic® F68 was used as a stabilizer. XRD analysis showed that these two crystalline forms belonged to the same polymorph with differences in molecular conformation and crystal size. It was demonstrated that the HPH process caused the partial transformation of DCF2 to DCF1 while no effect on DCF1 was observed. The change in the crystalline structure was attributed to the solubilization of DCF2 during HPH process and its subsequent recrystallization as the DCF1 form.

2.2.5. Stability issues with solidification process of nanosuspensions

When stable nanosuspensions are unattainable, the solid dosage form is the ultimate solution. The most common solidification processes are freeze drying and spray drying [10,19,20,118]. Since most solidified nanoparticle dry powders are usually reconstituted back into nanosuspensions during administration, drug nanocrystal growth or agglomeration during drying process needs to be prevented in order to maintain the nanosizing features such as rapid dissolution following the reconstitution. Adding matrix formers, such as mannitol, sucrose and cellulose, into nanosuspensions prior to drying is the common approach to overcome the stability issues during solidification process [19]. Since several excellent reviews have been published on this topic [19,25,118], the readers are directed to those reviews for more details.

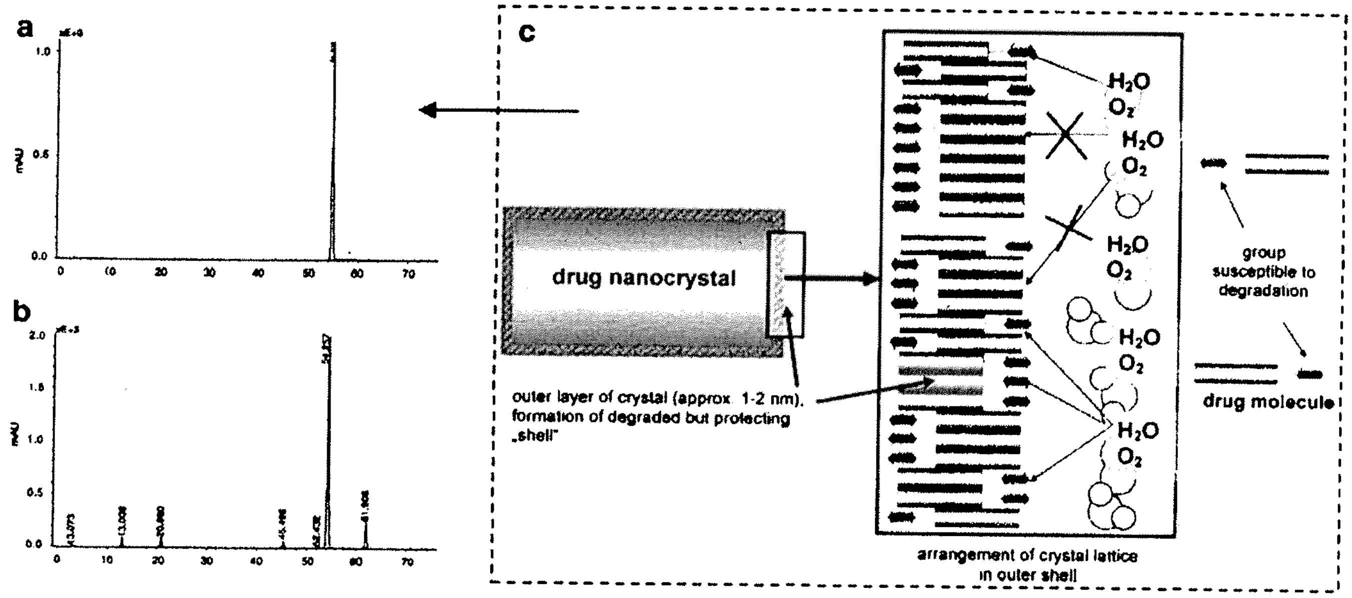
2.2.6. Chemical stability

Since drug nanocrystals are usually dispersed in nanosuspensions with a limited solubility, the possibility of chemical reactions is not as substantial as that in solution-based formulations. Consequently, chemical stability of nanosuspensions is generally superior to that of solutions. Paclitaxel serves as a good example to illustrate this [119]. Fig. 7(a) shows an HPLC diagram of paclitaxel nanosuspensions stabilized with Pluronic® F68 after 4 years of storage at 4–8 °C. No visible degradation product was observed with a recovery of more than 99%. On the other hand, paclitaxel solution with methanol as cosolvent showed clear degradation only after 48 h at room temperature (Fig. 7(b)). The excellent chemical stability of paclitaxel nanosuspensions was attributed to a mechanism similar to oxidized layer on the aluminum surface. Monolayer degradation on the nanocrystals surface was created once they were exposed to water and oxygen, as illustrated in Fig. 7(c). This monolayer could protect the inner part of drug crystals from further degradation, and thus enhance chemical stability of the nanosuspensions.

Unlike the physical stability issue that is a common concern for nanosuspensions, chemical stability is drug specific. Each molecule has its particular functional groups and reaction mechanism that affects the stability. For example, chemical functionalities, such as ester and amides, are susceptible to hydrolytic degradation, while amino groups may undergo oxidative degradation [120]. Although chemical stability of nanosuspensions is usually not a major concern, extra attention should be paid to drug molecules with solubility greater than 1 mg/mL or with low concentration in suspension [120]. The common strategy to enhance the chemical stability is to transform the nanosupensions into dry solid dosage form which is much more stable than nanosuspensions [19] or to increase the concentration of the nanosuspensions [120].

2.3. Additional stability issues relate to large biomolecules

Large biomolecules discussed in this review are mainly referred to therapeutic protein and peptide. The molecular structure of protein/peptide is distinctly different and more complicated as compared to that of the small molecules. The structures of large molecules are generally differentiated into four structures, i.e. primary, secondary, tertiary and quaternary structures [34]. These different structures refer to the sequence of the different amino acids, regions where the chains are organized into regular local structures by hydrogen bonding such as alpha helix and beta sheet, the mechanisms on how the protein/peptide chain folds into a 3-dimensional conformation, and the composition of multiple protein/peptide molecules assembly, respectively [32,33,123]. The intact molecular structure of protein/peptide is essential to maintain their therapeutic efficacy [35,121]. Common stability issues associated with protein/peptide include deamidation, oxidation, acylation, unfolding, aggregation and adsorption to surfaces [35,121]. These stability issues are affected by



Q1 Fig. 7. (a) HPLC diagram of paclitaxel aqueous nanosuspensions stabilized with Pluronic® F68; (b) HPLC diagram of paclitaxel solution (methanol: 10 ml, water: 5 ml, paclitaxel: 20.8 mg); (c) Schematic illustration of stabilization mechanism of paclitaxel nanosuspensions. Reprinted from Ref. [119] with permission from ELSEVIER.

temperature, solution pH, buffer ion, salt concentration, protein concentration, and added surfactants, with solution formulations being more susceptible to the influence from these factors than the suspension formulations [34,35,121]. Although suspension formulations or solid state of protein/peptide have enhanced stability due to their reduced molecular mobility, other stability issues may arise during particle formation or formulation process. For example, irreversible denaturation and aggregation upon reconstitution were often observed for dehydrated protein through freeze drying or spray-drying [125,126]. To prevent this, supplementary excipients such as bulking agents or surfactants are usually introduced during lyophilization [122].

The vulnerable structure of protein/peptide creates challenges for formulation development. Instead of using "naked" protein, the common strategy to prevent protein/peptide denaturation is to encapsulate the biomolecules with carrier such as liposome [123], SLN [124] or polymeric materials [125,126]. In addition to improving the stability, protein/peptide encapsulation can enhance bioavailability and provide sustained therapeutic release [125–128]. There has been plenty of work reported on encapsulated protein/peptide nanoparticles but very scarce studies on pure protein/peptide nanoparticles. Gomez et al. produced bovine zinc insulin nanoparticles using an electrospray drying technique and reported retained biological activities of the particles [129]. By using HPH, Maschke et al. attempted to micronize insulin in the medium of Myglyol 812 [130]. The stability and bioactivity of the insulin were maintained in spite of the harsh HPH process conditions. Merisko-Liversidge et al. [83] also noticed retained stability and bioactivity of zinc-insulin nanosuspensions that were produced through a wet milling process in presence of Pluronic® F68 and sodium deoxycholate. Nyambura et al. utilized a bottom up technique (combination of emulsification and freeze drying) to generate insulin nanoparticles (80 w/w% insulin with 20 wt.% lactose) [110]. The particles were then dispersed into HFA134a to produce an MDI formulation. The molecular integrity of insulin formulation, measured by HPLC, size exclusion chromatography, circular dichroism and fluorescence spectroscopy, indicated that native structures (primary, secondary and tertiary) were retained after particle formation and formulation process. The presence of surfactant (lecithin) and lyoprotectant (lactose) was believed to be responsible for preservation of the insulin structures. In their follow up work [109], they applied a similar approach to produce composite nanoparticles of lysozyme and lactose for MDI formulations. The retained biological activity of lysozyme was enhanced with increasing lactose concentration in the particles, and reached maximum (99% retained activity) with 20 w/w% lactose. Nanoprecipitation coupled with freeze drying was used as well in this work to produce spherical nanoparticles containing 80 w/w% lysozyme with fully preserved bioactivity. It was demonstrated that bioactivity of lysozyme nanoparticles remained unchanged when in contact with HFA 134a. Yu et al. compared the effectiveness of spray freezing into liquid (SFL) and spray-freeze drying (SFD) processes in producing bioactive lysozyme particles [131]. Both processes generated highly porous micro-sized aggregates of lysozyme primary nanoparticles in the size of 100–300 nm. SFL process was shown to produce lysozyme with lower aggregation and higher enzyme activity as compared to the SFD process, which was attributed to the shorter exposure time to the air–water interface during the SFL atomization process.

3. Characterizing stability of drug nanoparticles and nanoparticle formulations

Selection of characterization techniques for drug nanoparticles stability is dependent on the nature of stability issues and product dosage form. A few commonly used stability characterization techniques are listed in Table 2.

3.1. Particle size, size distribution and morphology

Particle size and size distribution are the key parameters used for evaluating the physical stability of nanoparticles. A variety of techniques, including photon correlation spectroscopy (PCS), also known as dynamic light scattering (DLS), laser diffraction (LD) and coulter counter, are commonly used to measure the particle size and size distribution (Table 2). The PCS/DLS is widely used to determine the size and size distribution of small particles suspended in liquid medium. The mean particle size and size distribution indicated as polydispersity index (PDI) are the typical measured parameters of this technique. A PDI value of 0.1 to 0.25 indicates a narrow size distribution while a PDI greater than 0.5 refers to a broad distribution [20]. Unfortunately, this technique is not capable of measuring size of dry powders and its measurement range is too narrow (3 nm to 3 μm) to detect the interference from the microparticles (>3 μm) within the

Table 2
Commonly used technique to evaluate the stability of nanoparticles.

Measured parameters	Techniques	Remarks
Particle size and size distribution	PCS/DLS	Pros: rapid, non-invasive. Cons: limited measurement range; apply only to liquid suspension.
	Laser diffraction	Pros: wide measurement range, rapid, non-invasive, apply to both liquid suspension and dry powder samples. Cons: particles are assumed to be spherical.
	Coulter counter	Pros: precise. Cons: apply only to spherical particles.
Particle size and morphology	SEM/TEM	Pros: evaluate both particle morphology and size, very small quantity of sample required. Cons: challenging to acquire statistical size distribution, usually invasive, time-consuming.
	AFM	Pros: non-invasive, evaluate both particle morphology and size, very small quantity of sample required. Cons: challenging to acquire statistical size distribution, time-consuming.
Sedimentation/creaming	Visual observation/laser backscattering/ near infrared transmission	–
Particle surface charge/zeta potential	Laser Doppler electrophoresis	–
Crystallinity state	XRD/DSC	–
Chemical stability	HPLC/FTIR/NMR/MS	–

nanosuspensions. Therefore, LD is often used in combination with PCS to circumvent this issue. Laser diffraction has a much wider detection range (20 nm to 2000 μm) and it can be used to evaluate both suspension and dry powder samples. The typical LD characterization parameters are LD50, LD90 and LD99, indicating 50, 90 or 99% of the particles are below the given size, respectively. LD is especially suitable for characterizing parenteral and pulmonary suspensions due to it wide measurement range. LD can detect the presence of microparticles (>5 μm) which are detrimental to parenteral nanosuspensions. However, LD provides only relative size distribution. The Coulter counter, on the other hand, measures the absolute number of particles per volume unit for the different size classes, and is more precise than the LD.

Although PCS, LD and coulter counter techniques provide rapid measurement of particle size and size distribution, they do not have the capability in evaluating particle morphology. As direct visualization techniques, Scanning Electron Microscope (SEM), Transmission Electron microscope (TEM) and Atomic Force Microscope (AFM) are widely used for assessment of particle morphology. However, it is very challenging and time-consuming to measure a significant number of particles to achieve statistical size distribution using these techniques. In addition, they usually require additional sample preparation such as coating that could be invasive to the particles, potentially causing some changes in particle properties.

3.2. Sedimentation/creaming

The traditional method to evaluate sedimentation or creaming is by visual observation over a period of time. By measuring the volume of the settled or creamed particle layer relative to the total suspension volume within a specific time, a dimensionless parameter known as sedimentation or flocculation volume can be obtained as a quantitative evaluation of suspension stability. A higher flocculation volume indicates a more stable suspension. The structure of settled/creamed layer can be easily assessed by re-dispersing the suspension, i.e. easily re-dispersed suspension indicates loose flocs while a dense cake is hard to be broken by manual shaking. Other approaches to evaluate sedimentation/creaming include laser backscattering [132] and near-infrared transmission [133].

3.3. Particle surface charge

Laser Doppler electrophoresis is commonly used to measure ZP. This technique evaluates electrophoretic mobility of suspended particles in the medium. It is a general rule of thumb that an absolute value of ZP above 60 mV yields excellent stability, while 30, 20 and less than 5 mV generally results in good stability, acceptable short-term stability and fast particle aggregation, respectively [29]. This rule of thumb is only valid for pure electrostatic stabilization or in combination with low-molecular weight surfactants, and is not valid when higher molecular weight stabilizers are present [29].

3.4. Crystalline state

The crystallinity of drug nanoparticles is usually assessed by X-Ray Diffraction (XRD) and/or Differential Scanning Calorimetry (DSC). XRD differentiates amorphous and crystalline nanoparticles as well as different polymorphic phases of the particles, while DSC is often used as a supplementary tool to XRD. Crystalline particles usually have a sharp melting peak which is absent in amorphous materials. The melting point can also be utilized to differentiate different polymorphs.

3.5. Chemical stability

HPLC is the most common characterization technique used to evaluate chemical stability that provides precise quantitative analysis on the degradation impurities. Mass spectrometry (MS) is often coupled with HPLC to identify the molecular structure of impurities. Some other techniques such as FTIR and NMR can also be used for chemical stability assessment. However, they are not as precise and sensitive as HPLC, and thus not widely used for stability assessment.

3.6. Additional techniques for assessing large biomolecule nanoparticle and formulation stability

For large biomolecules, additional characterization tools are generally required depending on the level of molecular structure to be assessed. For instance, size exclusion chromatography and electrophoresis are used to evaluate the primary structure of large biomolecules, circular dichroism is to monitor the secondary and tertiary structures while fluorescence spectroscopy is for tertiary structure [34,134]. In addition, *in-vitro* bioassays or *in-vivo* efficacy tests are needed to evaluate biological activities of the large biomolecules. Insulin particles, as an example, have been tested for its bioactivity either by *in-vitro* chondrocyte culture assays [130] or *in-vivo* monitoring of blood glucose level on rats following insulin administration [83].

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4. Recommendations of general strategies for enhancing stability of nanoparticle formulations

Strategies to address different stability issues are usually tailored according to different aspects, such as therapeutic requirements, dosage form and manufacturing complexity. For example, as the particle size is reduced, the sedimentation rate is decreased so that the particles can stay suspended longer in nanosuspensions. The general wisdom is that the smaller the nanoparticles are, the better. Unfortunately, too small particles are not always desirable, as they may create undesired plasma peaks due to the significant increases in dissolution rate [28]. Moreover, manufacturing complexity may be increased as well when the particles size requirements become too stringent.

The use of stabilizers is the most commonly used technique in achieving a stable nanoparticle formulation. However, the stabilizer selection is known to be very challenging. The challenge stems mainly from two aspects: (i) lack of fundamental understanding of interactions within nanosuspensions and (ii) lack of an efficient and high throughput stabilizer screening technique. In the case of aqueous nanosuspensions, it is relatively easy to select stabilizers given that water-based stabilizing moieties such as PEG and PVA are well known. However, selecting the anchor groups that interact strongly with the drug surface can be challenging due to the limited understanding on interactions between nanoparticles and stabilizers in molecular level. For non-aqueous nanosuspensions such as HFA-based MDI delivery system, understanding of solvation in the low-dielectric HFA medium is still in its infancy, which makes stabilizers selection even more challenging. Inefficient screening approaches are another hurdle for stabilizer selection. The current practice for stabilizer screening involves trial production of nanosuspensions with different stabilizers or stabilizer combinations, which could be burdensome and require vast amount of efforts especially with a large number of potential stabilizer candidates. AFM has recently been proven to be a feasible and efficient tool for stabilizer screening. Verma et al. demonstrated the feasibility of using AFM to select stabilizers for Ibuprofen nanosuspensions [135]. The AFM measurements showed that HPMC and HPC had extensive surface absorption on the ibuprofen surface, as opposed to the inadequate surface absorption with PVP and Pluronic® surfactants. These results correlated well with their stabilizing performances in the nanosuspensions. This finding confirmed the significance of AFM in providing a scientific rationale for stabilizer selection and improving understanding of the stabilization mechanisms. Another technique, known as colloidal probe microscopy (CPM) which is derived from AFM, has also been widely used to study interactions between colloidal particles and is expected to be a useful tool for nanosuspension stabilizer screening [136].

Due to the significant challenges associated with stabilizer selection, self-stabilized nanosuspensions with no added stabilizer are highly desirable. This is not only for simplifying the formulation development process but also reducing stabilizer-based toxicity. Unfortunately, the challenges to engineer such self-suspended nanoparticles are tremendous with very few reported studies to date. A couple of approaches that could potentially be used to produce self-stabilized nanosuspensions include the creation of drug nanoparticles with high ZP and controlling morphology or surface properties of drug nanoparticles to minimize inter-particulate forces.

5. Conclusions

The stability of drug nanoparticles remains a very challenging issue during pharmaceutical product development. Stability is affected by various factors such as dosage form (nanosuspension vs. dry solid), dispersion medium (aqueous vs. non-aqueous), delivery route (oral, inhalation, IV or other routes), production technique (top-down vs. bottom-up) and nature of drug (small molecules vs. large biomolecules). Despite the significant challenges associated with stabilizer screening, adding a stabilizer or combination of stabilizers is still the most commonly used and preferred approach to enhance the stability of nanosuspensions. Further understanding of particle-particle interactions within nanosuspensions and development of high-throughput stabilizer screening tools are essential to facilitate efficient stabilizer selection. Development of self-stabilized nanosuspensions, although currently seen as very complicated and challenging, is expected to grow with the continuing advancement in the field of particle engineering.

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EXHIBIT 5

US007217431B2

(12) **United States Patent**
Holm et al.

(10) **Patent No.: US 7,217,431 B2**
(45) **Date of Patent: May 15, 2007**

(54) **CONTROLLED AGGLOMERATION**

(75) Inventors: **Per Holm**, Vanløse (DK); **Anders Buur**, Allerød (DK); **Michiel Onne Elema**, Copenhagen (DK); **Birgitte Møllgaard**, Virum (DK); **Jannie Egeskov Holm**, Valby (DK); **Kirsten Schultz**, Roskilde (DK)

(73) Assignee: **LifeCycle Pharma A/S**, Horsholm (DK)

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(22) PCT Filed: **Jul. 5, 2002**

(86) PCT No.: **PCT/DK02/00472**

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(2), (4) Date: **Jul. 26, 2004**

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PCT Pub. Date: **Jan. 16, 2003**

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A61K 9/20 (2006.01)

(52) **U.S. Cl.** **424/474**; 424/78.08; 424/458; 424/459; 424/461; 424/464; 424/470; 424/497; 428/357; 428/402; 428/403; 428/407

(58) **Field of Classification Search** 428/357, 428/402, 403, 407; 424/78.08, 458, 459, 424/461, 464, 470, 474, 497
See application file for complete search history.

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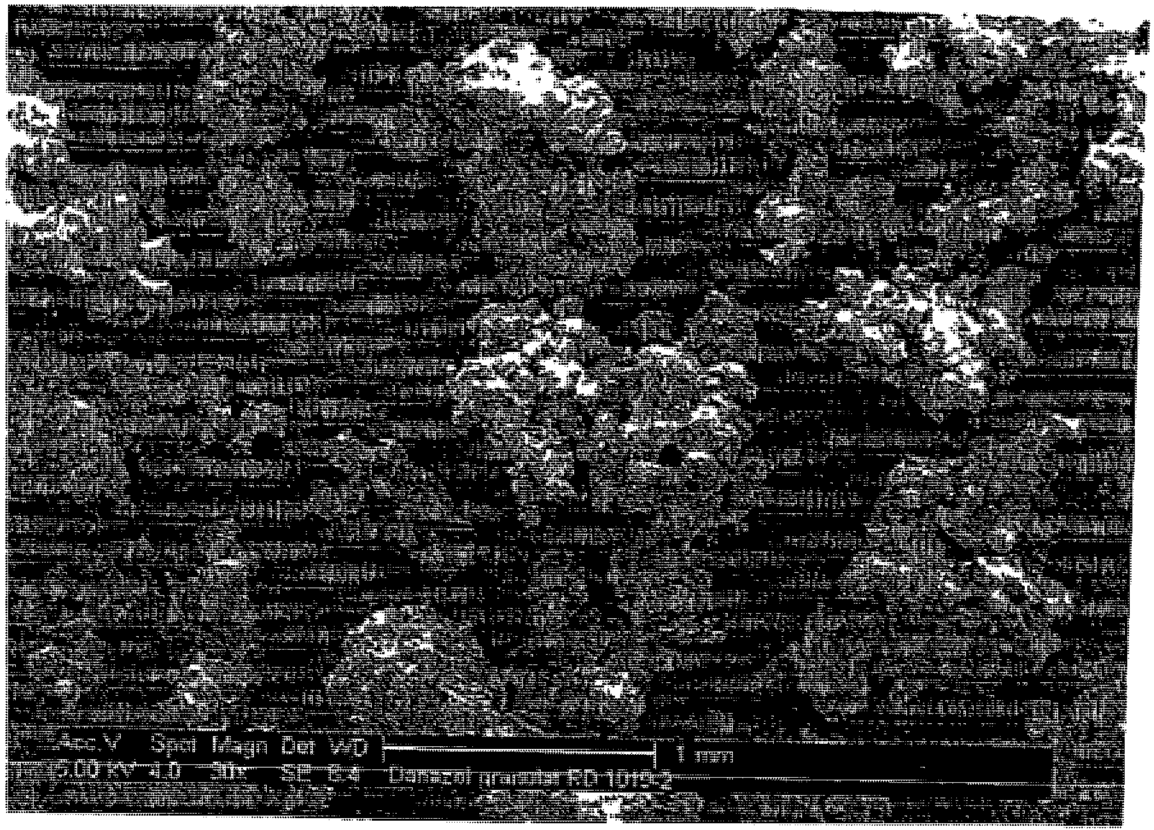
Primary Examiner—Irina S. Zemel
(74) *Attorney, Agent, or Firm*—Peter F. Corless; Stephana E. Patton; Edwards Angell Palmer & Dodge LLP

(57) **ABSTRACT**

A process for the preparation of a particulate material by a controlled agglomeration method, i.e. a method that enables a controlled growth in particle size. The method is especially suitable for use in the preparation of pharmaceutical compositions containing a therapeutically and/or prophylactically active substance which has a relatively low aqueous solubility and/or which is subject to chemical decomposition. The process comprising i) spraying a first composition comprising a carrier, which has a melting point of about 5° C. or more which is present in the first composition in liquid form, on a second composition comprising a material in solid form, the second composition having a temperature of at the most a temperature corresponding to the melting point of the carrier and/or the carrier composition and ii) mixing or others means of mechanical working the second composition onto which the first composition is sprayed to obtain the particulate material.

44 Claims, 8 Drawing Sheets



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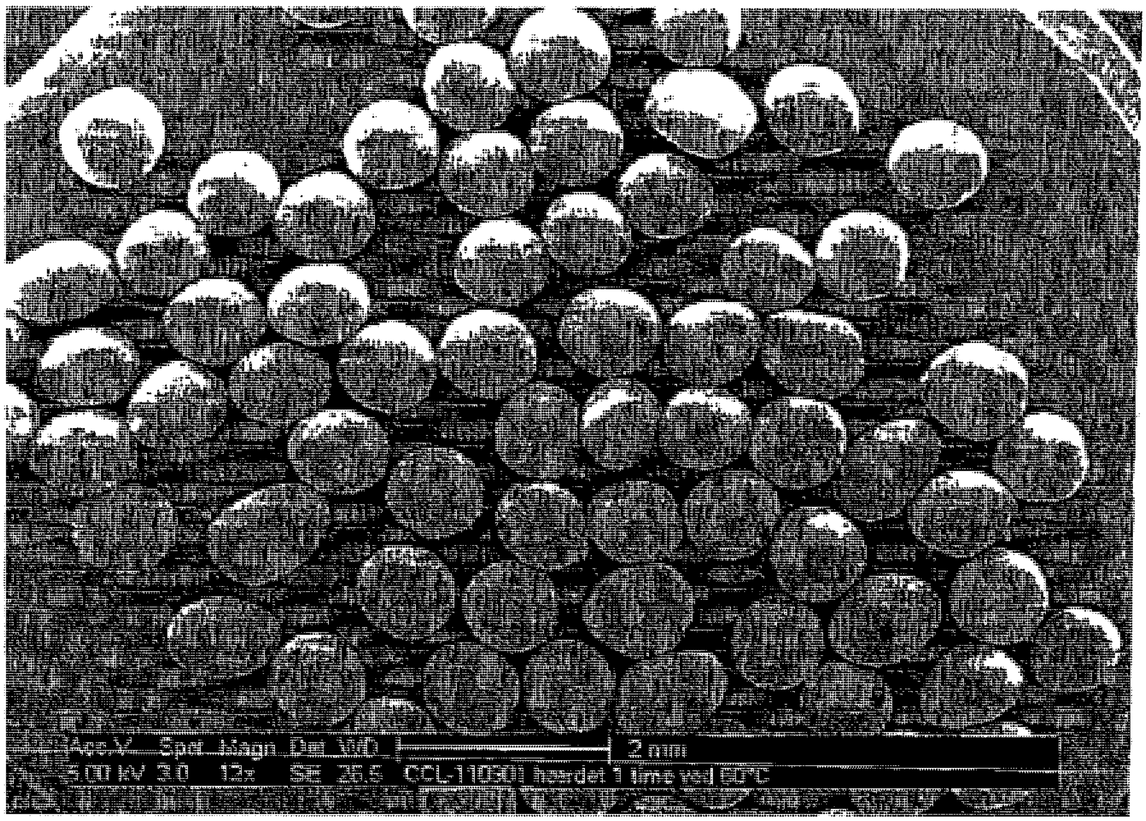

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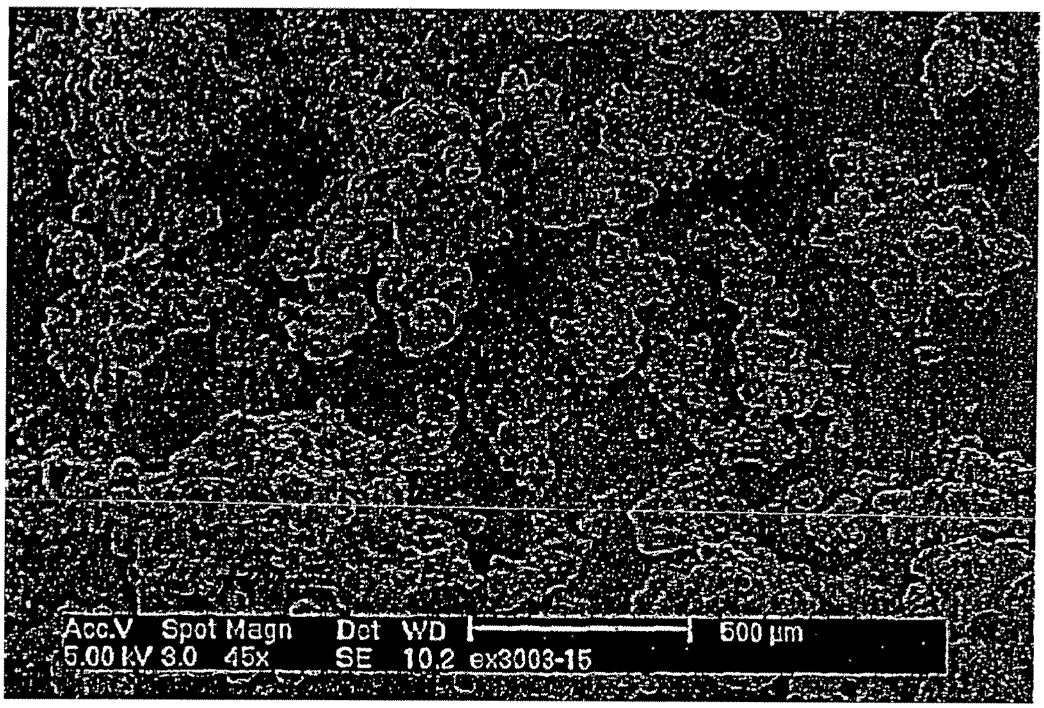


Fig. 3

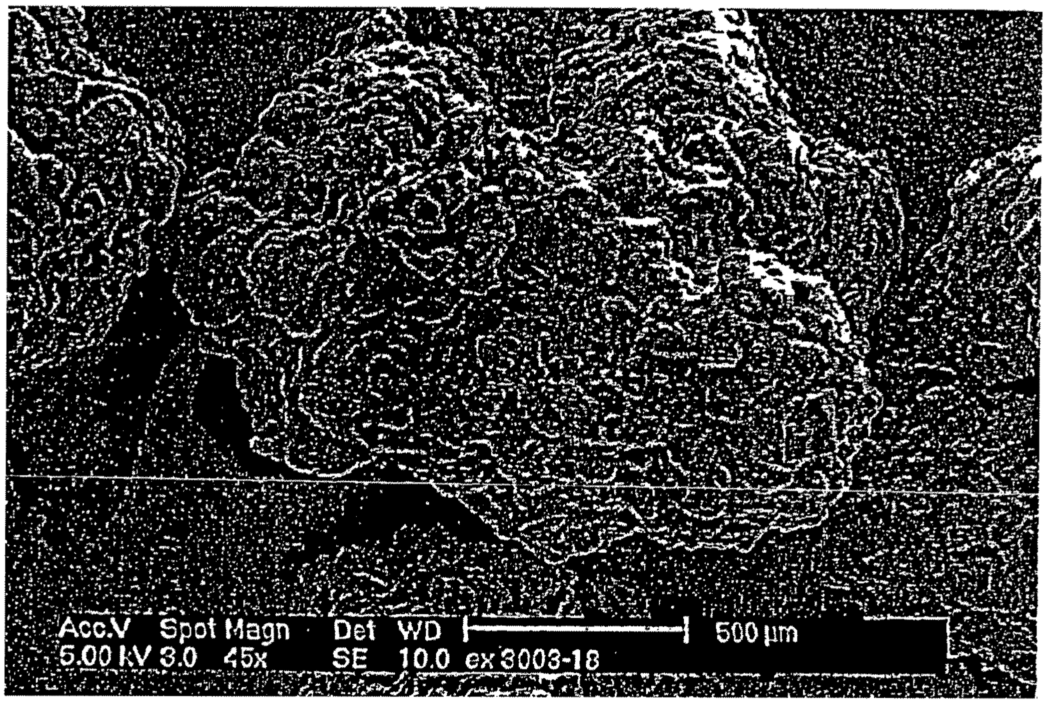


Fig. 4

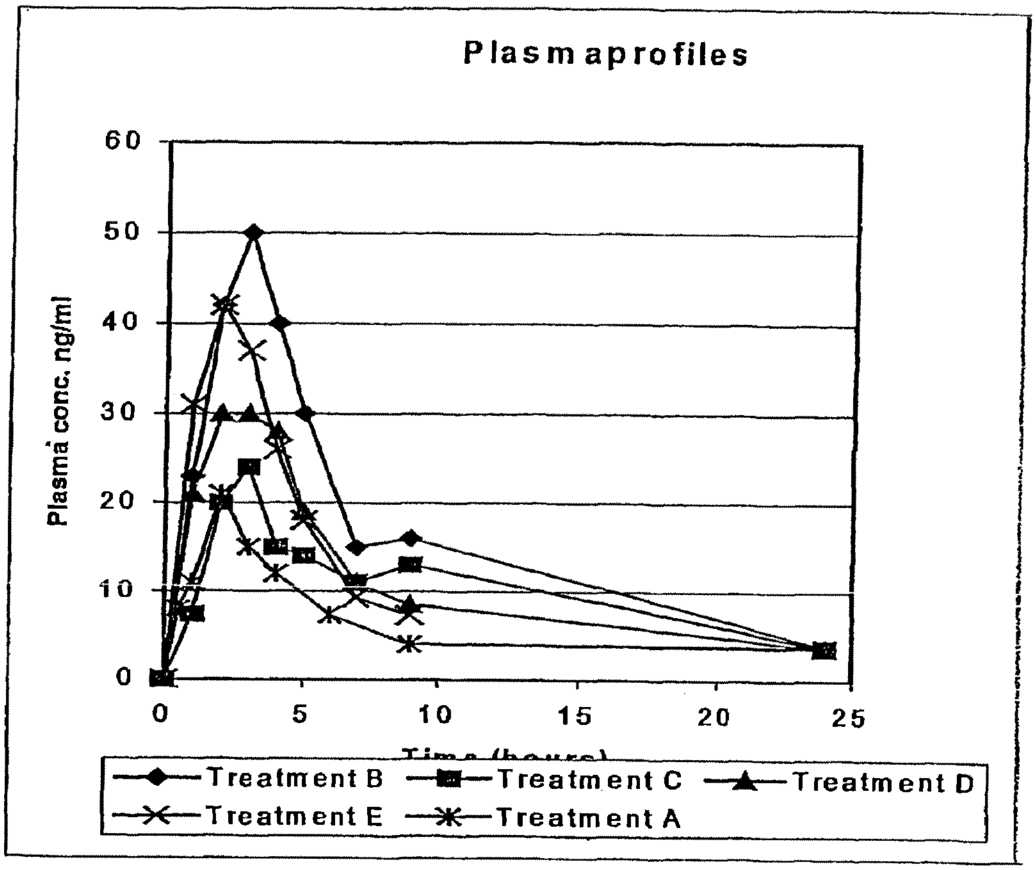

Plasmaprofiles
60
50
40
30
20
10
0
Plasma conc. ng/ml
0
5
10
15
20
25
Treatment B
Treatment C
Treatment D
Treatment E
Treatment A

Fig. 5

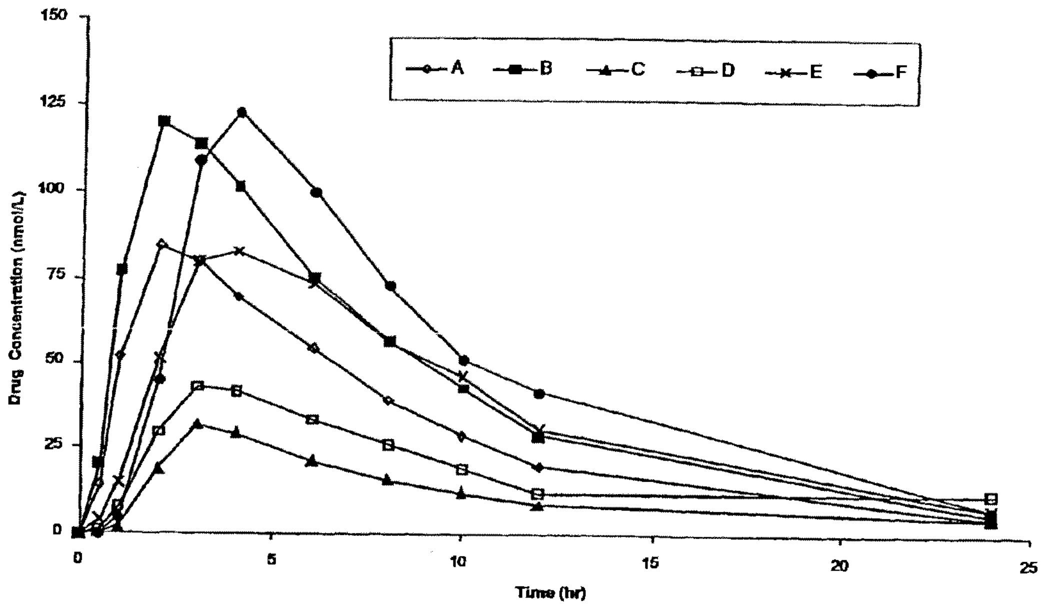
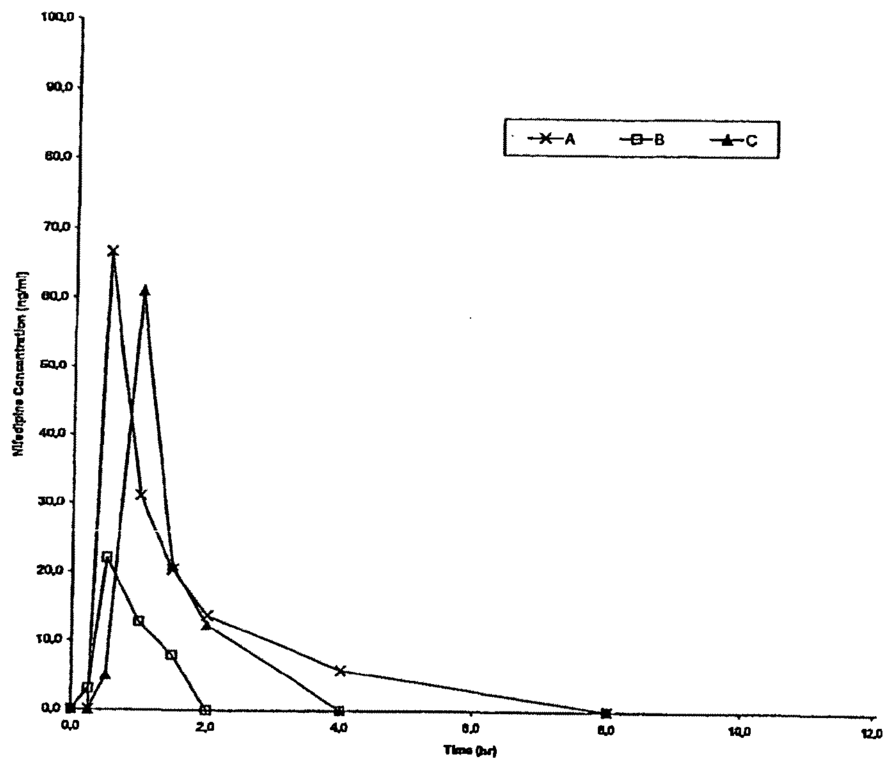

A
B
C
D
E
F
150
125
100
75
50
25
0
Drug Concentration (nmol/L)
0
5
10
15
20
25
Time (hr)

Fig. 6



Nifedipine Concentration (ng/ml)
Time (hr)
A
B
C
100,0
90,0
80,0
70,0
60,0
50,0
40,0
30,0
20,0
10,0
0,0
2,0
4,0
6,0
8,0
10,0
12,0

Fig. 7

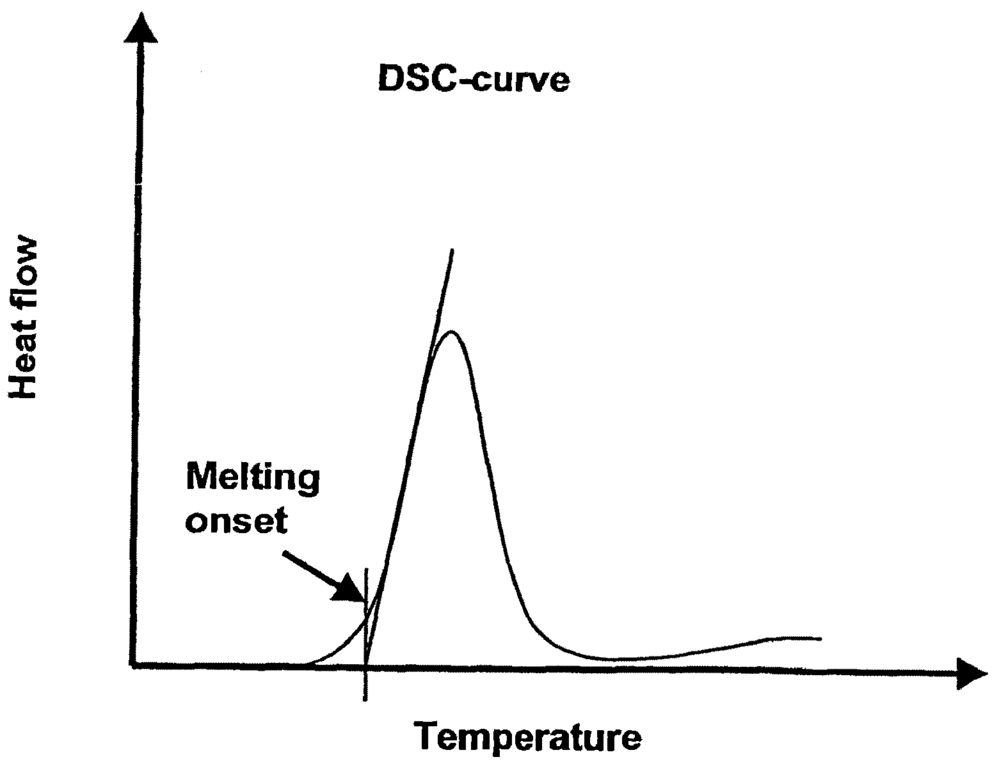


Fig 8

CONTROLLED AGGLOMERATION

FIELD OF THE INVENTION

The present invention relates to a process for the preparation of a particulate material by a controlled agglomeration method, i.e. a method that enables a controlled growth in particle size. The method is especially suitable for use in the preparation of pharmaceutical compositions containing a therapeutically and/or prophylactically active substance which has a relatively low aqueous solubility and/or which is subject to chemical decomposition. By employment of the novel process, compositions can be prepared that have improved properties with respect to release of the active substance from the composition as evidenced by in vitro dissolution test and/or with respect to improved shelf life of the compositions upon storage.

The invention also relates to a particulate material obtained by the novel process and to pharmaceutical compositions containing such particulate material. The particulate material obtained exhibits excellent flowability and compactability and possess excellent tabletting properties.

BACKGROUND OF THE INVENTION

There is a need for developing new and improved methods which enable preparation of pharmaceutical compositions for oral use that release the active substance from the composition in a suitable manner to enable an absorption of the active substance into the circulatory system.

DETAILED DISCLOSURE OF THE INVENTION

The present invention provides a method for controlled agglomeration, i.e. a controlled growth in particle size of a particulate material. Controlled agglomeration is provided using a process for the preparation of a particulate material (see below).

The invention also provides a process for the preparation of a particulate material, the process comprising

i) spraying a first composition comprising a carrier, which has a melting point of about 5° C. or more such as, e.g., about 10° C. or more, about 20° C. or more or about 25° C. or more and which is present in the first composition in liquid form, on a second composition comprising a material in solid form, the second composition having a temperature of at the most a temperature corresponding to the melting point of the carrier and/or of the carrier composition such as, e.g., a temperature of at least about 2° C., at least about 5° C. or at least about 10° C. lower than the melting point of the carrier and/or of the carrier composition, and

ii) mixing or other means of mechanical working the second composition onto which the first composition is sprayed to obtain the particulate material.

The process enables incorporation in a solid material of a high load of a carrier of a type that e.g. due to its solubility properties enables a high load of therapeutically and/or prophylactically active substances with a relatively low aqueous solubility. The carrier is normally solid or semi-solid and normally it has a sticky, oily or waxy character. However, the carrier may also be fluid at room temperature or even at temperature below 5° C. and in such cases it is contemplated that the process is carried out by employment of cooling of the second composition. By employment of the novel controlled agglomeration method a particulate material with a high load of carrier may be prepared and the resulting particulate material appears as a particulate powder in solid form. The particulate material obtained by the novel method has excellent properties with respect to flowability, bulk density, compactability and thus, it is suitable for use in the preparation of e.g. tablets. Although the particulate material may have a high load of a carrier of substantially sticky character the particulate material prepared has minimal, if any, adherence to tablet punches and/or dies during manufacture of tablets.

Methods for the preparation of granular products are described e.g. in EP-A-0 306 465 (Lejus Medical Aktiebolag), JP 60184378 (Takeda) and in WO 01/22941 (H. Lundbeck A/S). However, in none of these documents is described a method for the preparation of a particulate material, which method enables incorporation of a relatively high amount of a carrier as defined below and at the same time controlling the size of the particles obtained.

Carriers and Carrier Compositions

As indicated above an important step in the process for the preparation of a particulate material according to the invention is the addition of a carrier or a carrier composition. The carrier is of a type, which has a melting point of at least about 25° C. such as, e.g., at least about 30° C. at least about 35° C. or at least about 40° C. For practical reasons, the melting point may not be too high, thus, the carrier normally has a melting point of at the most about 300° C. such as, e.g., at the most about 250° C., at the most about 200° C., at the most about 150° C. or at the most about 100° C. If the melting point is higher then it becomes very difficult to ensure maintenance of a sufficient high temperature during the delivery of the carrier to the spraying equipment necessary to provide the melted carrier (or carrier composition) in the form of a spray. Furthermore, in those cases where e.g. a therapeutically and/or prophylactically active substance is included in the carrier composition, a relatively high temperature may promote e.g. oxidation or other kind of degradation of the substance.

In the present context, the melting point is determined by DSC (Differential Scanning Calorimetry). The melting point is determined as the temperature at which the linear increase of the DSC curve intersect the temperature axis (see FIG. **8** for further details).

Suitable carriers are generally substances, which are used in the manufacture of pharmaceuticals as so-called melt binders or solid solvents (in the form of solid dosage form), or as co-solvents or ingredients in pharmaceuticals for topical use.

The carrier may be hydrophilic, hydrophobic and/or they may have surface-active properties. In general hydrophilic and/or hydrophobic carriers are suitable for use in the manufacture of a pharmaceutical composition comprising a therapeutically and/or prophylactically active substance that has a relatively low aqueous solubility and/or when the release of the active substance from the pharmaceutical composition is designed to be immediate or non-modified. Hydrophobic carriers, on the other hand, are normally used in the manufacture of a modified release pharmaceutical composition. The above-given considerations are simplified to illustrate general principles, but there are many cases where other combinations of carriers and other purposes are relevant and, therefore, the examples above should not in any way limit the invention.

Examples on a suitable carrier are a hydrophilic carrier, a hydrophobic carrier, a surfactant or mixtures thereof.

Typically, a suitable hydrophilic carrier is selected from the group consisting of: polyether glycols such as, e.g.,

polyethylene glycols, polypropylene glycols; polyoxyethylenes; polyoxypropylenes; poloxamers and mixtures thereof, or it may be selected from the group consisting of: xylitol, sorbitol, potassium sodium tartrate, sucrose tribehenate, glucose, rhamnose, lactitol, behenic acid, hydroquinon monomethyl ether, sodium acetate, ethyl fumarate, myristic acid, citric acid, Gelucire 50/13, other Gelucire types such as, e.g., Gelucire 44/14 etc., Gelucire 50/10, Gelucire 62/05, Sucro-ester 7, Sucro-ester 11, Sucro-ester 15, maltose, mannitol and mixtures thereof.

A hydrophobic carrier for use in a process of the invention may be selected from the group consisting of: straight chain saturated hydrocarbons, sorbitan esters, paraffins; fats and oils such as e.g., cacao butter, beef tallow, lard, polyether glycol esters; higher fatty acid such as, e.g. stearic acid, myristic acid, palmitic acid, higher alcohols such as, e.g., cetanol, stearyl alcohol, low melting point waxes such-as, e.g., glyceryl monostearate, hydrogenated tallow, myristyl alcohol, stearyl alcohol, substituted and/or unsubstituted monoglycerides, substituted and/or unsubstituted diglycerides, substituted and/or unsubstituted triglycerides, yellow beeswax, white beeswax, carnauba wax, castor wax, japan wax, acetylate monoglycerides; NVP polymers, PVP polymers, acrylic polymers, or a mixture thereof.

In an interesting embodiment, the carrier is a polyethylene glycol having an average molecular weight in a range of from about 400 to about 35,000 such as, e.g., from about 800 to about 35,000, from about 1,000 to about 35,000 such as, e.g., polyethylene glycol 1,000, polyethylene glycol 2,000, polyethylene glycol 3,000, polyethylene glycol 4,000, polyethylene glycol 5,000, polyethylene glycol 6000, polyethylene glycol 7,000, polyethylene glycol 8,000, polyethylene glycol 9,000 polyethylene glycol 10,000, polyethylene glycol 15,000, polyethylene glycol 20,000, or polyethylene glycol 35,000. In certain situations polyethylene glycol may be employed with a molecular weight from about 35,000 to about 100,000.

In another interesting embodiment, the carrier is polyethylene oxide having a molecular weight of from about 2,000 to about 7,000,000 such as, e.g. from about 2,000 to about 100,000, from about 5,000 to about 75,000, from about 10,000 to about 60,000, from about 15,000 to about 50,000, from about 20,000 to about 40,000, from about 100,000 to about 7,000,000 such as, e.g., from about 100,000 to about 1,000,000, from about 100,000 to about 600,000, from about 100,000 to about 400,000 or from about 100,000 to about 300,000.

In another embodiment, the carrier is a poloxamer such as, e.g. Poloxamer 188, Poloxamer 237, Poloxamer 338 or Poloxamer 407 or other block copolymers of ethylene oxide and propylene oxide such as the Pluronic® and/or Tetronic® series. Suitable block copolymers of the Pluronic® series include polymers having a molecular weight of about 3,000 or more such as, e.g. from about 4,000 to about 20,000 and/or a viscosity (Brookfield) from about 200 to about 4,000 cps such as, e.g., from about 250 to about 3,000 cps. Suitable examples include Pluronic® F38, P65, P68LF, P75, F77, P84, P85, F87, F88, F98, P103, P104, P105, F108, P123, F123, F127, 10R8, 17R8, 25R5, 25R8 etc. Suitable block copolymers of the Tetronic series include polymers having a molecular weight of about 8,000 or more such as, e.g., from about 9,000 to about 35,000 and/or a viscosity (Brookfield) of from about 500 to about 45,000 cps such as, e.g., from about 600 to about 40,000. The viscosities given above are determined at 60° C. for substances that are pastes at room temperature and at 77° C. for substances that are solids at room temperature.

The carrier may also be a sorbitan ester such as, e.g., sorbitan di-isostearate, sorbitan dioleate, sorbitan monolaurate, sorbitan monoisostearate, sorbitan monooleate, sorbitan monopalmitate, sorbitan monostearate, sorbitan sesquiisostearate, sorbitan sesquioleate, sorbitan sesquistearate, sorbitan tri-isostearate, sorbitan trioleate, sorbitan tristearate or mixtures thereof.

The carrier composition may of course comprise a mixture of different carriers such as, e.g., a mixture of hydrophilic and/or hydrophobic carriers.

In another interesting embodiment, the carrier is a surfactant or a substance having surface-active properties. It is contemplated that such substances are involved in the wetting of e.g. slightly soluble active substance and thus, contributes to improved solubility characteristics of the active substance.

Examples on surfactants are given in the following. In order to be suitable for use as a carrier, the criteria with respect to melting point and/or viscosity discussed herein must be fulfilled. However, the list below encompasses surfactants in general, because surfactants may also be added to the carrier composition in the form of pharmaceutically acceptable excipients.

In a process according to the invention, the carrier may be employed as such or in the form of a carrier composition. A carrier composition comprises one or more carriers optionally together with one or more other ingredients. Thus, the carrier composition may comprise a mixture of hydrophilic and/or hydrophobic carriers and/or surfactants. The carrier composition may also comprise one or more therapeutically and/or prophylactically active substances and/or one or more pharmaceutically acceptable excipients.

Suitable excipients for use in a carrier composition (and—as discussed above—for use as carriers it selves) are surfactants such as, e.g., hydrophobic and/or hydrophilic surfactants as those disclosed in WO 00/50007 in the name of Lipocine, Inc. Examples on suitable surfactants are

i) polyethoxylated fatty acids such as, e.g. fatty acid mono- or diesters of polyethylene glycol or mixtures thereof such as, e.g. mono- or diesters of polyethylene glycol with lauric acid, oleic acid, stearic acid, myristic add, ricinoleic acid, and the polyethylene glycol may be selected from PEG 4, PEG 5, PEG 6, PEG 7, PEG 8, PEG 9, PEG 10, PEG 12, PEG 15, PEG 20, PEG 25, PEG 30, PEG 32, PEG 40, PEG 45, PEG 50, PEG 55, PEG 100, PEG 200, PEG 400, PEG 600, PEG 800, PEG 1000, PEG 2000, PEG 3000, PEG 4000, PEG 5000, PEG 6000, PEG 7000, PEG 8000, PEG 9000, PEG 1000, PEG 10,000, PEG 15,000, PEG 20,000, PEG 35,000,
ii) polyethylene glycol glycerol fatty acid esters, i.e. esters like the above-mentioned but in the form of glyceryl esters of the individual fatty acids;
iii) glycerol, propylene glycol, ethylene glycol, PEG or sorbitol esters with e.g. vegetable oils like e.g. hydrogenated castor oil, almond oil, palm kernel oil, castor oil, apricot kernel oil, olive oil, peanut oil, hydrogenated palm kernel oil and the like,
iv) polyglycerized fatty acids like e.g. polyglycerol stearate, polyglycerol oleate, polyglycerol ricinoleate, polyglycerol linoleate,
v) propylene glycol fatty acid esters such as, e.g. propylene glycol monolaurate, propylene glycol ricinoleate and the like,
vi) mono- and diglycerides like e.g. glyceryl monooleate, glyceryl dioleae, glyceryl mono- and/or dioleate, glyceryl caprylate, glyceryl caprate etc.;

vii) sterol and sterol derivatives;

viii) polyethylene glycol sorbitan fatty acid esters (PEG-sorbitan fatty acid esters) such as esters of PEG with the various molecular weights indicated above, and the various Tween® series;

ix) polyethylene glycol alkyl ethers such as, e.g. PEG oleyl ether and PEG lauryl ether,

x) sugar esters like e.g. sucrose monopalmitate and sucrose monolaurate;

xi) polyethylene glycol alkyl phenols like e.g. the Triton® X or N series:

xii) polyoxyethylene-polyoxypropylene block copolymers such as, e.g., the Pluronic® series, the Synperonic® series, Emkalyx®, Lutrol®, Supronic® etc. The generic term for these polymers is "poloxamers" and relevant examples in the present context are Poloxamer 105, 108, 122, 123, 124, 181, 182, 183, 184, 185, 188, 212, 215, 217,231, 234, 235, 237, 238, 282, 284, 288, 331, 333, 334, 335, 338, 401, 402,403 and 407;

xiii) sorbitan fatty acid esters like the Span® series or Ariacel® series such as, e.g. sorbinan monolaurate, sorbitan monopalmitate, sorbitan monooleate, sorbitan monostearate etc.;

xiv) lower alcohol fatty acid esters like e.g. oleate, isopropyl myristate, isopropyl palmitate etc.;

xv) ionic surfactants including cationic, anionic and zwitterionic surfactants such as, e.g. fatty acid salts, bile salts, phospholipids, phosphoric acid esters, carboxylates, sulfates and sulfonates etc.

When a surfactant or a mixture of surfactants is present in a carrier composition the concentration of the surfactant(s) is normally in a range of from about 0.1–75% w/w such as, e.g., from about 0.1 to about 20% w/w, from about 0.1 to about 15% w/w, from about 0.5 to about 10% w/w, or alternatively, when applicable as a carrier or a part of the carrier composition from about 20 to about 75% w/w such as, e.g. from about 25 to about 70% w/w, from about 30 to about 60% w/w.

Other suitable excipients in a carrier composition may be solvents or semi-solid excipients like, e.g. propylene glycol, polyglycolised glycerides including Gelucire 44/14, complex fatty materials of plant origin including theobroma oil, carnauba wax, vegetable oils like e.g. almond oil, coconut oil, corn oil, cottonseed oil, sesame oil, soya oil, olive oil, castor oil, palm kernels oil, peanut oil, rape oil, grape seed oil etc., hydrogenated vegetable oils such as, e.g. hydrogenated peanut oil, hydrogenated palm kernels oil, hydrogenated cottonseed oil, hydrogenated soya oil, hydrogenated castor oil, hydrogenated coconut oil; natural fatty materials of animal origin including beeswax, lanolin, fatty alcohols including cetyl, stearyl, lauric, myristic, palmitic, stearic fatty alcohols; esters including glycerol stearate, glycol stearate, ethyl oleate, isopropyl myristate; liquid interesterified semi-synthetic glycerides including Miglycol 810/812; amide or fatty acid alcolamides including stearamide ethanol, diethanolamide of fatty coconut acids etc.

Other additives in the carrier composition may be antioxidants like e.g. ascorbic acid, ascorbyl palmitate, butylated hydroxyanisole, butylated hydroxytoluene, hypophosphorous acid, monothioglycerol, potassium metabisulfite, propyl gallate, sodium formaldehylde sulfoxylate, sodium metabisulfite, sodium thiosulfate, sulfur dioxide, tocopherol, tocopherol acetate, tocopherol hemisuccinate, TPGS or other tocopherol derivatives, etc. The carrier composition may also contain e.g. stabilising agents. The concentration of an antioxidant and/or a stabilizing agent in the carrier composition is normally from about 0.1% w/w to about 5% w/w.

In those cases where a carrier composition is employed, the requirements with respect to the melting point mentioned above normally also apply to the carrier composition, especially in those cases where a minor amount of water is included in the carrier composition. However, when the carrier composition is heated the carrier composition may be in the form of two or more phases (e.g. two distinct liquid phase, or a liquid phase comprising e.g. an active substance dispersed therein). In such cases, the melting point is not a true melting point but merely a heating point where the carrier composition becomes in a liquid form, which is suitable for use in a spraying device. Often such a heating point will for practical purposes correspond to the melting point of the carrier itself.

The total concentration of carrier(s) in the carrier composition is normally in a range of from about 5 to about 100% w/w such as, e.g., from about 10 to about 99.5% w/w, from about 15 to about 99% w/w, from about 15 to about 98% w/w, from about 15 to about 97% w/w, from about 20 to about 95% w/w such as at least about 25% w/w, at least about 30% w/w, at least about 35% w/w, at least about 40% w/w, at least about 45% w/w, at least about 50% w/w, at least about 55% w/w, at least about 60% w/w, at least about 65% w/w, at least about 70% w/w, at least about 75% w/w, at least about 80% w/w, at least about 85% w/w, at least about 90% w/w, at least about 95% w/w or at least about 98% w/w.

As explained above, in a process according to the invention the carrier or the carrier composition is brought on liquid form by heating the carrier and/or the carrier composition to a temperature, which causes the carrier and/or the carrier composition to melt, and the carrier in liquid form (i.e. as a solution or a dispersion) is sprayed on the second composition.

As mentioned above, the carrier or the carrier composition in melted or liquidized form is sprayed on a second composition. Thus, the carrier or the carrier composition should have a suitable viscosity. If the viscosity is too high, the carrier or carrier composition will be too "thick" and will have a tendency of adhering to the nozzle, which may result in that the delivery through the nozzle is stopped. For the present purpose a viscosity of the carrier and/or the carrier composition is suitably if the viscosity (Brookfield DV-III) is at the most about 800 mPas at a temperature of at the most 100° C. such as, e.g., at the most 700, at the most 600, at the most 500 mPas. In those cases where the melting point of the carrier or the carrier composition is more than about 80° C., the viscosity values mentioned above are at a temperature of about 40° C. above the melting point.

In the particulate material obtained by a process according to the invention, the concentration of the carrier is from about 5 to about 95% w/w such as, e.g. from about 5 to about 90% w/w, from about 5 to about 85% w/w, from about 5 to about 80% w/w, from about 10 to about 75% w/w, from about 15 to about 75% w/w, from about 20 to abut 75% w/w, from about 25% to about 75% w/w, from about 30% to about 75% w/w. from about 35% to about 75% w/w, from about 25% to about 70% w/w, from about 30% to about 70% w/w, from about 35% to abut 70% w/w. from about 40% to about 70% w/w, from about 45% to about 65% w/w or from about 45% to about 60% w/w.

In those cases where the second composition comprises a pharmaceutically acceptable excipient that has a relatively high particle density it is preferred that the concentration of the carrier in the particulate material obtained by a process

of the invention is from about 5 to about 95% v/v such as, e.g. from about 5 to about 90% v/v, from about 5 to about 85% v/v, from about 5 to about 80% v/v, from about 10 to about 75% v/v from about 15 to about 75% v/v, from about 20 to abut 75% v/v, from about 25% to about 75% v/v, from about 30% to about 75% v/v, from about 35% to about 75% v/v, from about 25% to about 70% v/v, from about 30% to about 70% v/v, from about 35% to abut 70% v/v, from about 40% to about 70% v/v, from about 45% to about 65% v/v or from about 45% to about 60% v/v.

In the following is given a calculation example:

Recalculation from % w/w to % vv (of total composition):

Particle density of lactose: 1.56 g/cm^3

Particle density of calcium hydrogen phosphate anhydrous: 2.89 g/cm^3

Particle density of PEG 6000: 1.17 g/cm^3

For lactose: w/w ratio of 50% PEG 6000/(lactose+PEG 6000) equals a % v/v of 56% For calcium hydrogen phosphate anhydrous: w/w ratio of 50% PEG 6000/(calcium hydrogen phosphate anhydrous+PEG 6000) equals a % v/v of 71%.

In many cases it is suitable to dissolve or disperse a therapeutically and/or prophylactically active substance in the carrier or in the carrier composition. Suitable therapeutically and/or prophylactically active substances are discussed below.

In a process according to the invention it is not necessary to employ water or an aqueous medium e.g. together with a binder in order to build up agglomerates of a suitable size. The agglomeration suitably takes place under water-free or substantially water-free conditions. Thus, the process is also very useful when active substances or other ingredients are employed which are susceptible to water (e.g. degradation under aqueous conditions). However, if desired, water or an aqueous medium may of course be incorporated in the carrier composition. Although the carrier composition normally is essentially non-aqueous, water may be present to a certain extent and then the concentration of water in the carrier composition is the most about 20% w/w water such as at the most about 15% w/w, at the most abut 10% w/w, at the most about 5% w/w or at the most about 2.5% w/w.

Therapeutically and/or Prophylactically Active Substances

In a preferred embodiment of the invention the particulate material obtained by a process according to the invention comprises a therapeutically and/or prophylactically active substance. The particulate matter may also or alternatively comprise a cosmetically active substance (i.e. a substance that is employed in cosmetic compositions). In a process according to the invention the active substance may be included in the carrier composition and/or in the second composition.

In the present context a therapeutically and/or prophylactically active substance includes any biologically and/or physiologically active substance that has a function on an animal such as, e.g. a mammal like a human. The term includes drug substances, hormones, genes or gene sequences, antigen-comprising material, proteins, peptides, nutrients like e.g. vitamins, minerals, lipids and carbohydrates and mixtures thereof. Thus, the term includes substances that have utility in the treatment and/or preventing of diseases or disorders affecting animals or humans, or in the regulation of any animal or human physiological condition. The term also includes any biologically active substance which, when administered in an effective amount, has an effect on living cells or organisms.

Many active substances have and it is expected that many of the future drug substances will have undesired properties especially with respect to water solubility and to oral bioavailability. Therefore, a novel technology, which enables especially therapeutically and/or prophylactically active substances to be delivered to the body in a relatively easy manner and at the same time enables the desired therapeutic and/or prophylactic response, is highly needed.

By employment of a process according to the present invention it is contemplated that this object can be achieved for many such substances, especially in view of the promising results the inventors have obtained from a study in Beagle dogs. Accordingly, the present inventors have found very promising results with respect to bioavailability when a process according to the invention is employed for the preparation of particulate material containing an active substance with a very low aqueous solubility. Thus, a process according to the invention is especially suitable for use for the preparation of particulate material comprising an active substance that has an aqueous solubility at 25° C. and pH of 7.4 of at the most about 3 mg/ml such as, e.g., at the most about 2 mg/ml, at the most about 1 mg/ml, at the most about 750 μg/ml, at the most about 500 μg/ml, at the most about 250 μg/ml, at the most about 100 μg/ml, at the most about 50 μg/ml, at the most about 25 μg/ml, at the most about 20 μg/ml or at the most about 10 μg/ml. In specific embodiments the solubility of the active substance may be much lower such as, e.g., at the most about 1 μg/ml, at the most about 100 μg/ml, at the most about 75 μg/ml such as about 50 μg/ml.

As mentioned above a process according to the invention may advantageously be carried out without employment of water or an aqueous medium. Thus, the process is especially suitable for use for active substances that are degraded, decomposed or otherwise influenced by water.

Examples on active substances suitable for use in a particulate material according to the invention are in principle any active substance such as, e.g. freely water soluble as well as more slightly or insoluble active substances. Thus, examples on active substances suitable for use are e.g. antibacterial substances, antihistamines and decongestants, anti-inflammatory agents, antiparasitics, antivirals, local anesthetics, antifungals, amoebicidals or trichomonocidal agents, analgesics, antianxiety agents, anticlotting agents, antiarthritics, antiasthmatics, antiarthritic, anticoagulants, anticonvulsants, antidepressants, antidiabetics, antiglaucoma agents, antimalarials, antimicrobials, antineoplastics, antiobesity agents, antipsychotics, antihypertensives, antitussives, auto-immune disorder agents, anti-impotence agents, anti-Parkinsonism agents, anti-Alzheimers' agents, antipyretics, anticholinergics, anti-ulcer agents, anorexic, beta-blockers, beta-2 agonists, beta agonists, blood glucose-lowering agents, bronchodilators, agents with effect on the central nervous system, cardiovascular agents, cognitive enhancers, contraceptives, cholesterol-reducing agents, cytostatics, diuretics, germicidals, H-2 blockers, hormonal agents, hypnotic agents, inotropics, muscle relaxants, muscle contractants, physic energizers, sedatives, sympathomimetics, vasodilators, vasoconstrictors, tranquilizers, electrolyte supplements, vitamins, counterirritants, stimulants, anti-hormones, drug antagonists, lipid-regulating agents, uricosurics, cardiac glycosides, expectorants, purgatives, contrast materials, radiopharmaceuticals, imaging agents, peptides, enzymes, growth factors, etc.

Specific examples include e.g.

Anti-inflammatory drugs like e.g. ibuprofen, indometacin, naproxen, nalophine;

Anti-Parkinsonism agents like e.g. bromocriptine, biperidin, benzhexol, benztropine etc.

Antidepressants like e.g. imipramine, nortriptyline, pritiptyline, etc.

Antibiotics like e.g. clindamycin, erythomycin, fusidic acid, gentamicin, mupirocine, amfomycin, neomycin, metronidazol, sulphamethizole, bacitracin, framycetin, polymyxin B, acitromycin etc,

Antifungal agents like e.g. miconazol, ketoconaxole, clotrimazole, amphotericin B, nystatin, mepyramin, econazol, fluconazol, flucytocine, griseofulvin, bifonazole, amorofine, mycostatin, itrconazole, terbenafine, terconazole, tolnaftate etc.

Antimicrobial agents like e.g. metronidazole, tetracyclines, oxytetracylines, peniciilins etc.

Antiemetics like e.g. metoclopramide, droperidol, haloperidol, promethazine etc.

Antihistamines like e.g. chlorpheniramine, terfenadine, triprolidine etc.

Antimigraine agents like e.g. dihydroergotamine, ergotamine, pizofylline etc.

Coronary, cerebral or peripheral vasodilators like e.g. nifedipine, diltiazem etc.

Antianginals such as, e.g., glyceryl nitrate, isosorbide dinitrate, molsidomine, verapamil etc.

Calcium channel blockers like e.g. verapamil, nifedipine, diltiazem, nicardipine etc.

Hormonal agents like e.g. estradiol, estron, estriol, polyestradiol, polyestriol, dienestrol, diethylstilbestrol, progesterone, dihydroprogesterone, cyprosterone, danazol, testosterone etc.

Contraceptive agents like e.g. ethinyl estradiol, lynestrenol, etynodiol, norethisterone, mestranol, norgestrel, levonorgestrel, desodestrel, medroxyprogesterone etc.

Antithrombotic agents like e.g. heparin, warfarin etc.

Diuretics like e.g. hydrochlorothiazide, flunarizine, minoxidil etc.

Antihypertensive agents like e.g. propanolol, metoprolol, clonidine, pindolol etc.

Corticosteroids like e.g. beclomethasone, betamethasone, betamethasone-17-valerate, betamethasone-dipropionate, clobetasol, clobetasol-17-butyrate, clobetasol-propionate, desonide, desoxymethasone, dexamethasone, diflucortolone, flumethasone, flumethasone-pivalte, fluocinolone acetonide, fluocinoide, hydrocortisone, hydrocortisone-17-butyrate, hydrocortisonebuteprate, methylprednisolone, triamcinolone acetonide, hacinonide, fluprednide acetate, alklometasone-dipropionate, fluocortolone, fluticasonpropionte, mometasone-furate, desoxymethasone, difluorason-diacetate, halquinol, cliochinol, chlorchinaldol, fluocinolone-acetonide etc.

Dermatological agents like e.g. nitrofurantoin, dithranol, clioquinol, hydroxyquinoline, isotretionin, methoxsalen, methotrexate, tretionin, trioxalen, salicylic add, penicillamine etc.

Steroids like e.g. estradiol, progesterone, norethindrone, levonorgestrel, ethynodiol, levonorgestrol, norgestimate, gestanin, desogestrel, 3-keton-desogesterel, demegestone, promethoestrol, testosterone, spironolactone and esters thereof etc.

Nitro compounds like e.g. amyl nitrates, nitroglycerine and isosorbide nitrate etc.

Opioids like e.g. morphine, buprenorphine, oxymorphone, hydromorphone, codeine, tramadol etc.

Prostaglandins such as, e.g., a member of the PGA, PGB, PGE or PGF series such as, e.g. minoprostol, dinoproston, carboprost, eneprostil etc.

Peptides like e.g. growth hormone releasing factors, growth factors (e.g. epidermal growth factor (EGF), nerve growth factor (NGF), TGF, PDGF, insulin growth factor (IGF), fibroblast growth factor (aFGF, bFGF etc.), somatostatin, calcitonin, insulin, vasopressin, interferons, IL-2 etc., urokinase, serratiopeptidase, superoxide dismutase, thyrotropin releasing hormone, lutenizing hormone releasing hormone (LH-RH), corticotrophin releasing hormone; growth hormone releasing hormone (GHRH), oxytodin, erythropoietin (EPO), colony stimulating factor (CSF) etc.

Interesting examples on active substances that are slightly soluble, sparingly soluble or insoluble in water are given in the following tables:

TABLE 1

Poorly-Soluble Drug Candidates

Drug Name	Therapeutic Class	Solubility In Water
Alprazolam	CNS	Insoluble
Amiodarone	Cardiovascular	Very Slightly
Amlodipine	Cardiovascular	Slightly
Astemizole	Respiratory	Insoluble
Atenolol	Cardiovascular	Slightly
Azathioprine	Anticancer	Insoluble
Azelastine	Respiratory	Insoluble
Beclomethasone	Respiratory	Insoluble
Budesonide	Respiratory	Sparingly
Buprenorphine	CNS	Slightly
Butalbital	CNS	Insoluble
Carbamazepine	CNS	Insoluble
Carbidopa	CNS	Slightly
Cefotaxime	Anti-infective	Sparingly
Cephalexin	Anti-infective	Slightly
Cholestyramine	Cardiovascular	Insoluble
Ciprofloxacin	Anti-infective	Insoluble
Cisapride	Gastrointestinal	Insoluble
Cisplatin	Anticancer	Slightly
Clarithromycin	Anti-infective	Insoluble
Clonazepam	CNS	Slightly
Clozapine	CNS	Slightly
Cyclosporin	Immunosuppressant	Practically Insoluble
Diazepam	CNS	Slightly
Diclofenac sodium	NSAID	Sparingly
Digoxin	Cardiovascular	Insoluble
Dipyridamole	Cardiovascular	Slightly
Divalproex	CNS	Slightly
Dobutamine	Cardiovascular	Sparingly
Doxazosin	Cardiovascular	Slightly
Enalapril	Cardiovascular	Sparingly
Estradiol	Hormone	Insoluble
Etodolac	NSAID	Insoluble
Etoposide	Anticancer	Very Slightly
Famotidine	Gastrointestinal	Slightly
Felodipine	Cardiovascular	Insoluble
Fentanyl citrate	CNS	Sparingly
Fexofenadine	Respiratory	Slightly
Finasteride	Genito-urinary	Insoluble
Fluconazole	Antifungal	Slightly
Flunosolide	Respiratory	Insoluble
Flurbiprofen	NSAID	Slightly
Fluvoxamine	CNS	Sparingly
Furosemide	Cardiovascular	Insoluble
Glipizide	Metabolic	Insoluble
Glyburide	Metabolic	Sparingly
Ibuprofen	NSAID	Insoluble
Isosorbide dinitrate	Cardiovascular	Sparingly
Isotretinoin	Dermatological	Insoluble
Isradipine	Cardiovascular	Insoluble
Itraconzole	Antifungal	Insoluble
Ketoconazole	Antifungal	Insoluble
Ketoprofen	NSAID	Slightly
Lamotrigine	CNS	Slightly

TABLE 1-continued

Poorly-Soluble Drug Candidates		
Drug Name	Therapeutic Class	Solubility In Water
Lansoprazole	Gastrointestinal	Insoluble
Loperamide	Gastrointestinal	Slightly
Loratadine	Respiratory	Insoluble
Lorazepam	CNS	Insoluble
Lovastatin	Cardiovascular	Insoluble
Medroxyprogesterone	Hormone	Insoluble
Mefenamic acid	Analgesic	Slightly
Methylprednisolone	Steroid	Insoluble
Midazolam	Anesthesia	Insoluble
Mometasone	Steroid	Insoluble
Nabumetone	NSAID	Insoluble
Naproxen	NSAID	Insoluble
Nicergoline	CNS	Insoluble
Nifedipine	Cardiovascular	Practically Insoluble
Norfloxacin	Anti-infective	Slightly
Omeprazole	Gastrointestinal	Slightly
Paclitaxel	Anticancer	Insoluble
Phenytoin	CNS	Insoluble
Piroxicam	NSAID	Sparingly
Quinapril	Cardiovascular	Insoluble
Ramipril	Cardiovascular	Insoluble
Risperidone	CNS	Insoluble
Saquinavir	Protease inhibitor	Practically Insoluble
Sertraline	CNS	Slightly
Simvastatin	Cardiovascular	Insoluble
Terbinafine	Antifungal	Slightly
Terfenadine	Respiratory	Slightly
Triamcinolone	Steroid	Insoluble
Valproic acid	CNS	Slightly
Zolpidem	CNS	Sparingly

TABLE 2

Poorly-Soluble Drugs with Low Bioavailability			
Drug Name	Indication	Solubility In Water	Bioavailability
Astemizole	Allergic Rhinitis	Insoluble	Low–moderate
Cyclandelate	Peripheral vascular disease	Insoluble	Low
Perphenazine	Psychotic disorder	Insoluble	Low
Testosterone	Androgen Replacement Therapy	Insoluble	Low
Famotidine	GERD	Slightly soluble	Low (39–50%)
Budesonide	Allergic Rhinitis	Sparingly soluble	Low (~15%)
Mesalamine	Irritable Bowel Syndrome	Slightly soluble	Low (~20%)
Clemastine fumarate	Allergic Rhinitis	Slightly soluble	Low (~39%)
Buprenorphine	Pain	Slightly soluble	Low (<30%)
Sertraline	Anxiety	Slightly soluble	Low (<44%)
Auranofin	Arthritis	Slightly soluble	Low (15–25%)
Felodipine	Hypertension	Insoluble	Low (15%)
Isradipine	Hypertension	Insoluble	Low (15–24%)
Danazol	Endometriosis	Insoluble	Low
Loratadine	Allergic Rhinitis	Insoluble	Low
Isosorbide dinitrate	Angina	Sparingly soluble	Low (20–35%)
Fluphenazine	Psychotic disorder	Insoluble	Low (2–3%)
Spironolactone	Hypertension, Edema	Insoluble	Low (25%)
Biperiden	Parkinson's disease	Sparingly soluble	Low (29–33%)
Cyclosporin	Transplantation	Slightly soluble	Low (30%)
Norfloxacin	Bacterial Infection	Slightly soluble	Low (30–40%)
Cisapride	GERD	Insoluble	Low (35–40%)
Nabumetone	Arthritis	Insoluble	Low (35%)
Dronabinol	ANTIEMETIC	Insoluble	Low 10–20%)
Lovastatin	Hyperlipidemia	Insoluble	Low (~5%)
Simvastatin	Hyperlipidemia	Insoluble	Low (<5%)

The amount of active substance incorporated in a particulate material (and/or in a pharmaceutical, cosmetic or food composition) may be selected according to known principles of pharmaceutical formulation. In general, the dosage of the active substance present in a particulate material according to the invention depends inter alia on the specific drug substance, the age and condition of the patient and of the disease to be treated.

A particulate material according to the invention may comprise a cosmetically active ingredient and/or a food ingredient. Specific examples include vitamins, minerals, vegetable oils, hydrogenated vegetable oils, etc.

Second Composition

As mentioned above the carrier or carrier composition is sprayed on a second composition. In order to be able to achieve a high amount of carrier in the final particulate material and in order to enable a controlled agglomeration of the particles comprised in the second composition, the present inventors have surprisingly found that in specific embodiments, the second composition should initially have a temperature which is at least about 10° C. such as, e.g., at least about 15° C., at least about 20° C., at least about 25° C., or at least about 30° C. below the melting point of the carrier or carrier composition (or, as discussed above, the heating point of the carrier composition). However, as mentioned above, a temperature difference of at least about 10° C. it is not always necessary. Thus, the second composition may have a temperature of at the most a temperature corresponding to the melting point of the 2° C., at least about 5° C. No external heating of the second composition is normally employed during the process of the invention, but in some cases it may be advantageous to employ a cooling

via the inlet air. However, the temperature of the second composition may increase to a minor extent due to the working of the composition. However, the temperature must (or will) not be higher than at the most the melting point of the carrier or carrier composition such as, e.g. at the most about 5° C. such as at the most about 10° C., at the most about 15° C. or at the most about 20° C. below the melting point of the carrier or the carrier composition. Accordingly, a process of the invention can be carried out without any heating of the second composition, i.e. it can be carried out at ambient or room temperature (i.e. normally in a range of from about 20° C. to about 25° C.).

In contrast thereto, known melt granulation methods involve external heating of the material that is to be granulated (or agglomerated) together with a melt binder.

The second composition comprises pharmaceutically and/or cosmetically acceptable excipients and, furthermore, a therapeutically and/or prophylactically active substance may be present in the second composition.

In the present context the terms "pharmaceutically acceptable excipient" and "cosmetically acceptable excipient" are intended to denote any material, which is inert in the sense that i substantially does not have any therapeutic and/or prophylactic effect per se. Such an excipient may be added with the purpose of making it possible to obtain a pharmaceutical and/or cosmetic composition, which has acceptable technical properties.

Examples on suitable excipients for use in a second composition include fillers, diluents, disintegrants, binders, lubricants etc. or mixture thereof. As the particulate material obtained by a process according to the invention may be used for different purposes, the choice of excipients is normally made taken such different uses into considerations. Other pharmaceutically acceptable excipients for use in a second composition (and/or in the carrier composition) are e.g. acidifying agents, alkalizing agents. preservatives, antioxidants, buffering agents, chelating agents, coloring agents, complexing agents, emulsifying and/or solubilizing agents, flavors and perfumes, humectants, sweetening agents, wetting agents etc.

Examples on suitable fillers, diluents and/or binders include lactose (e.g. spray-dried lactose, α-lactose, β-lactose, Tabletose®, various grades of Pharmatose®, Microtose® or Fast-Floc®), microcrystalline cellulose (various grades of Avicel®, Elcema®, Vivacel®, Ming Tai® or Solka-Floc®), hydroxypropylcellulose, L-hydroxypropylcellulose (low substituted), hydroxypropyl methylcellulose (HPMC) (e.g. Methocel E, F and K, Metolose SH of Shin-Etsu, Ltd, such as, e.g. the 4,000 cps grades of Methocel E and Metolose 60 SH, the 4,000 cps grades of Methocel F and Metolose 65 SH, the 4,000, 15,000 and 100,000 cps grades of Methocel K; and the 4,000, 15,000, 39,000 and 100,000 grades of Metolose 90 SH), methylcellulose polymers (such as, e.g., Methocel A, Methocel A4C, Methocel A15C, Methocel A4M), hydroxyethylcellulose, sodium carboxymethylcellulose, carboxymethylene, carboxymethylhydroxyethylcellulose and other cellulose derivatives, sucrose, agarose, sorbitol, mannitol, dextrins, maltodextrins, starches or modified starches (including potato starch, maize starch and rice starch), calcium phosphate (e.g. basic calcium phosphate, calcium hydrogen phosphate, dicalcium phosphate hydrate), calcium sulfate, calcium carbonate, sodium alginate, collagen etc.

Specific examples of diluents are e.g. calcium carbonate, dibasic calcium phosphate, tribasic calcium phosphate, calcium sulfate, microcrystalline cellulose, powdered cellulose, dextrans, dextrin, dextrose, fructose, kaolin, lactose, mannitol, sorbitol, starch, pregelatinized starch, sucrose, sugar etc.

Specific examples of disintegrants are e.g. alginic acid or alginates, microcrystalline cellulose, hydroxypropyl cellulose and other cellulose derivatives, croscarmellose sodium, crospovidone, polacrillin potassium, sodium starch glycolate,.starch, pregelatinized starch, carboxymethyl starch (e.g. Primogel® and Explotab®) etc.

Specific examples of binders are e.g. acacia, alginic acid, agar, calcium carrageenan, sodium carboxymethylcellulose, microcrystalline cellulose, dextrin, ethylcellulose, gelatin, liquid glucose, guar gum, hydroxypropyl methylcellulose, methylcellulose, pectin, PEG, povidone, pregelatinized starch etc.

Glidants and lubricants may also be included in the second composition. Examples include stearic acid, magnesium stearate, calcium stearate or other metallic stearate, talc, waxes and glycerides, light mineral oil, PEG, glyceryl behenate, colloidal silica, hydrogenated vegetable oils, corn starch, sodium stearyl fumarate, polyethylene glycols, alkyl sulfates, sodium benzoate, sodium acetate etc.

Other excipients which may be included in the second composition (and/or in the carrier composition) are e.g. colouring agents, taste-masking agents, pH-adjusting agents, solubilizing agents, stabilising agents, wetting agents, surface active agents, antioxidants, agents for modified release etc.

In certain cases it may be advantageously to incorporate a magnesium aluminometasilicate in the particulate material. It may be a part of the second composition or it may be added subsequently in order to facilitate a further processing of the particulate material (e.g. to prepare solid dosage forms like capsules or tablet). Magnesium aluminometasilicate is sold under the name Neusilin and is obtainable from Fuji Chemical Industries. Neusilin is normally used in order to improve filling capacity and compression property of powders and granules when added. Neusilin is also believed to reduce weight variation and to improve hardness and disintegration of tablets. Finally, Neusilin has an adsorption capability, which makes it suitable for use when processing waxy materials like oil extracts and waxes into pharmaceutical composition. Especially Neusilin UFL2 and US2 are said to be suitable for such a use.

Thus, in one aspect the invention relates to a process, wherein the second composition comprises magnesium aluminosilicate and/or magnesium aluminometasilicate such as, e.g, Neusilin S1, Neusilin FH2, Neusilin US2, Neusilin UFL2 or the like. Other suitable substances are contemplated to be bentonite, kaolin, magnesium trisilicate, montmorillonite and/or saponite. In a still further embodiment, the second composition comprises magnesium aluminosilicate and/or magnesium aluminometasilicate such as, e.g, Neusilin, and the particulate material obtained has an content of carrier of at least about 30% vv such as, e.g, at least about 40% v/v, at Yeast about 50% v/v, at least about 60% v/v, at least about 70% v/v, at least about 75% v/v, at least about 80% v/v, at least about 85% v/v or at least about 90% v/v.

Besides the known use of Neusilin, the present inventors have found that specific qualities of magnesium aluminometasilicate (Neusilin) have excellent properties as glidants or anti-adhesive most likely due to the porous structure of Neusilin. Thus, Neusilin may advantageously be added in order to reduce any adherence of the particulate material to the manufacturing equipment in particular to the tabletting machine. In the examples herein is given a com-

parison of the anti-adhesive properties of Neusilin compared with known lubricants and Neusilin seems to be a very promising and novel candidate as a lubricant.

Details on Controlled Agglomeration

A process according to the invention may be carried out in a high or low shear mixer or in a fluid bed. Important characteristics are that the carrier or the carrier composition is sprayed on the second composition, which is loaded into the mixer or the fluid bed. Normally, the carrier or the carrier composition is heated to a temperature above the melting point of the carrier and/or the carrier composition and the second composition has not been subject to any heating and has normally ambient temperature. The difference in temperature between the carrier and the second composition makes the carrier solidify rapidly which in turn leads to a controlled growth of the particle size. Thus, the inventors have found that by employing such conditions it is possible to control the agglomeration process so that the growth in particle size is controlled.

In the present context, the term "controlled agglomeration" is intended to mean that the increase in mean geometric diameter of a material is a linear or approximated linear function of the carrier concentration in the carrier composition (see FIG. 1). Controlled agglomeration is also present if a d_{gw} of < or =500 μm is obtained when a carrier composition containing 20% carrier has been added to a second composition.

The possibility of controlling the agglomeration makes it possible to obtain a particulate material that has a very high load of carrier(s)—much higher than described when conventional methods like e.g. melt granulation is employed. As discussed above, a high load of carrier has shown to be of importance especially when particulate material is prepared containing a slightly water-soluble, sparingly water soluble or insoluble active substances. FIG. 2 is a theoretically calculated curve showing the relationship between obtainable dose and drug solubility in a carrier composition at different carrier concentrations in the particulate material assuming a total composition weight of 500 mg. It is seen that the dose can be increased by a factor of about 3.5 by increasing the concentration of carrier from 20% to 70%. By conventional melt granulation, i.e. a process by which heating of a melt binder and excipients is performed, normally a load of at the most about 15% w/w of the melt binder is obtained (calculated on the final composition). Another granulation method, which makes use of the same temperature of the binder and the material to be granulated, is a conventional granulation process, which is performed either by a wet or a dry granulation process.

A SEM micrograph in FIG. 3 shows a particulate material prepared by a process according to the present invention. PEG 6000 is used as a carrier and lactose is used as the second composition. The figure shows that the primary particles of lactose are agglomerated by immersion in the droplets of PEG 6000 or by coalescence between larger agglomerates. The agglomerates are partly coated with PEG 6000. The probability of agglomerate growth by coalescence is reduced by rapidly solidifying PEG due to the product temperature being kept at a minimum of 10° C. below the melting point of PEG.

In contrast thereto, uncontrolled agglomeration is shown in a SEM micrograph in FIG. 4. The particulate material is prepared according to Example 2 herein (uncontrolled agglomeration) using PEG 6000 as carrier and lactose as excipients. The figure shows that the particulate material has larger agglomerates with surplus of liquefied PEG at the surface of the agglomerates increasing the probability of agglomerate growth by coalescence at elevated product temperature.

A process according to the invention may be carried out in a fluid bed. In such cases the second composition is normally kept in a fluidized state by incoming air at ambient temperature. The carrier or carrier composition is sprayed on the fluidized second composition and in order to keep the carrier or carrier composition on a liquid form and/or to avoid any clotting of the spraying device, the spraying device is kept at a suitable temperature above the melting point of the carrier or carrier composition. Normally, the spraying is performed through a spraying device equipped with temperature controlling means.

The particulate material obtained by a process of the invention has a geometric weight mean diameter d_{gw} of ≧10 μm such as, e.g, 220 μm, from about 20 to about 2000, from about 30 to about 2000, from about 50 to about 2000, from about 60 to about 2000, from about 75 to about 2000 such as, e.g. from about 100 to about 1500 μm, from about 100 to about 1000 μm or from about 100 to about 700 μm. In specific embodiments the geometric weight mean diameter d_{gw} is at the most about 400 μm or at the most 300 μm such as, e.g., from about 50 to about 400 μm such as, e.g., from about 50 to about 350μm, from about 50 to about 300 μm, from about 50 to about 250 μm or from about 100 to about 300 μm.

Particulate Material—Characteristics

Many characteristics of the particulate material obtained by a process according to the invention have already been discussed. In summary, a particulate material has good tabletting properties including good flowability and compactability. It has no or minimal adherence to the tabletting equipment either in itself or after addition of the normal amount of lubricants. It is an excellent alternative for incorporation of active substances with very low water solubility and/or with a very low bioavailability, or active substances, which are subject to degradation in the presence of water (the process may be carried out without any water).

Thus, a particulate material of the invention is excellent for a further processing into e.g. tablets. In contrast to capsules, tablets are normally easier and cheaper to produce and tablets are often preferred by the patient. Furthermore, a tablet formulation is relatively easy to adjust to specific requirements, e.g. with respect to release of the active substance, size etc.

The particulate material may also be coated (see Examples) with a film coating, an enteric coating, a modified release coating, a protective coating, an anti-adhesive coating etc.

Suitable coating materials are e.g. methylcellulose, hydroxypropylmethylcellulose, hydroxypropylcellulose, acrylic polymers, ethylcellulose, cellulose acetate phthalate, polyvinyl acetate phthalate, hydroxypropyl methylcellulose phthalate, polyvinylalcohol, sodium carboxymethylcellulose, cellulose acetate, cellulose acetate phthalate, gelatin, methacrylic acid copolymer, polyethylene glycol, shellac, sucrose, titanium dioxide, carnauba wax, microcrystalline wax, zein.

Plasticizers and other ingredients may be added in the coating material. The same or different active substance may also be added in the coating material.

Pharmaceutical Compositions

The particulate material obtained by a process according to the invention may be used as such or it may be further processed to the manufacture of a pharmaceutical and/or a

cosmetic composition by addition of one or more suitable pharmaceutically and/or cosmetically acceptable excipients. Furthermore, the particulate material obtained may be provided with a coating to obtain coated particles, granules or pellets. Suitable coatings may be employed in order to obtain composition for immediate or modified release of the active substance and the coating employed is normally selected from the group consisting of film-coatings (for immediate or modified release) and enteric coatings or other kinds of modified release coatings, protective coatings or anti-adhesive coatings

The particulate material obtained by a process of the invention is especially suitable for further processing into tablets. The material possesses suitable properties for tabletting purposes, cf. below, but in some cases it may be suitable to add further therapeutically and/or prophylactically active substances and/or excipients to the particulate material before the manufacture of tablets. For examples, by using a mixture of i) an active substance contained in modified release coated granules or granules in the form of modified release matrices and ii) an active substance in freely accessible form, a suitable release pattern can be designed in order to obtain a relatively fast release of an active substance followed by a modified (i.e. often prolonged) release of the same or a different active substance.

As appears from the above, a particulate material obtained by a process of the invention is suitable for use in the manufacture of tablets obtained by direct compression. Furthermore, the particulate material may in itself be employed as a binding agent for use in dry granulation processes.

A particulate material obtained by a process according to the invention may be employed in any kind of pharmaceutical compositions in which the use of a solid particulate material is applicable. Thus, relevant pharmaceutical compositions are e.g. solid, semi-solid, fluid or liquid composition or compositions in the form of a spray. The particulate material may also be incorporated in a suitable drug delivery device such as, e.g. a transdermal plaster, a device for vaginal use or an implant.

Solid compositions include powders, and compositions in dosage unit form such as, e.g. tablets, capsules, sachets, plasters, powders for injection etc.

Semi-solid compositions include compositions like ointments, creams, lotions, suppositories, vagitories, gels, hydrogels, soaps, etc.

Fluid or liquid compositions include solutions, dispersions such as, e.g., emulsions, suspension, mixtures, syrups, etc.

Accordingly, the invention also relates to any pharmaceutical composition comprising a particulate material obtainable by a process of the invention.

Other Aspects of the Invention

The invention also relates to a pharmaceutical particulate material obtained by mixing a first and a second composition as defined herein and heating to a temperature that is below the melting point of a carrier contained in the first composition. The heating may be applied while mixing or in a separate step. The particulate material generally has a geometric weight mean diameter d_{gw} of ≧10 μm such as, e.g. ≧20 μm, from about 20 to about 2000, from about 30 to about 2000, from about 50 to about 2000, from about 60 to about 2000, from about 75 to about 2000 such as, e.g. from about 100 to about 1500 μm, from about 100 to about 1000 μm or from about 100 to about 700 μm, or at the most about 400 μm or at the most 300 μm such as, e.g., from about 50 to about 400 μm such as, e.g., from about 50 to about 350 μm, from about 50 to about 300 μm, from about 50 to about 250 μm or from about 100 to about 300 μm. In such a material the concentration of the carrier typically is at least about 40% v/v.

Such a particulate material is especially suitable for use in the preparation of solid dosage form such as tablets, capsules, sachets and the like. It may have sufficient properties with respect to flowability and/or anti-adhesion so that addition of e.g. a lubricant can be omitted when preparing a solid dosage form, especially if it comprises magnesium aluminosilicate and/or magnesium aluminometasilicate.

In a further aspect, the invention relates to the use of magnesium aluminosilicate and/or magnesium aluminometasilicate as a lubricant.

All details described herein for the main aspect of the invention apply *mutatis mutandi* to any other aspect of the invention.

LEGENDS TO FIGURES

FIG. **1** shows the correlation between amount of PEG 6000 sprayed onto lactose 125 mesh and mean granule size (geometric weight mean diameter) for a product temperature of 40–45° C. and 50–60 ° C., respectively. The dashed line indicates uncontrolled agglomeration at a PEG concentration of approx. 25% at a product temperature of 50–60° C. The products are unscreened.

FIG. **2** shows the relationship between obtainable dose and drug solubility in a carrier at different concentrations of carrier assuming a formulation unit weight of 500 mg.

FIG. **3** is a SEM micrograph of PEG sprayed onto lactose 125 mesh; the PEG concentration is 48% w/w. Magnification×45.

FIG. **4** is a SEM micrograph of PEG sprayed onto lactose 125 mesh; the PEG concentration is 25% w/w. Magnification×45.

FIG. **5** shows results from Example 4.

FIG. **6** shows mean serum concentrations vs. time profiles after p.o. administration of the model drug substance from Example 5 (30 mg) in six different formulations to Beagle dogs. Treatment A: 0.5% HPC (aq.), Treatment B: 5% Captisol® (aq.), Treatment C: Model drug substance from Example 5/SLS (2:1), Treatment D: Model drug substance from Example 5/SLS (1:1), Treatment E: Tween 80, Kollidon VA64, corn starch and lactose, Treatment F: Akosoft® 3103.

FIG. **7** shows the plasma concentration versus time curves for formulation A, B, C described in Example 6 after oral administration to dogs.

FIG. **8** illustrates determination of a melting point by a DSC curve.

The invention is further illustrated in the following examples.

Methods

Determination of Weight Variation

The tablets prepared in the Examples herein were subject to a test for weight variation performed in accordance with Ph. Eur.

Determination of Average Tablet Hardness

The tablets prepared in the Examples herein were subject to at test for tablet hardness employing Schleuniger Model 6D apparatus and performed in accordance with the general instructions for the apparatus.

Determination of Disintegration Time

The time for a tablet to disintegrate, i.e. to decompose into particles or agglomerates, was determined in accordance with Ph. Eur.

Determination of Geometric Weight Mean Diameter d_{gw}

The geometric weight mean diameter was determined by employment of a method of laser diffraction dispersing the particulate material obtained (or the starting material) in air. The measurements were performed at 1 bar dispersive pressure in Sympatec Helos equipment, which records the distribution of the equivalent spherical diameter. This distibution is fitted to a log normal volume-size distribution.

When used herein, "geometric weight mean diameter" means the mean diameter of the log normal volume-size distribution.

Determination of Aqueous Solubility

The aqueous solubility at 25° C. in distilled or purified water was determined by suspending a well-defined and excessive amount of the substance under investigation in a well-defined amount of distilled or purified water. The dispersion is stirred and samples are withdrawn after suitable time periods. The samples are filtered and the filtrate analysed to obtain the concentration of the substance in the sample. The concentration of the substance in the sample is then calculated according to methods well known for a person skilled in the art. The solubility is reached when the concentrations of the substance in two consecutive samples are considered identical.

Determination of Dissolution Rate

The dissolution rate was determined by employment of USP paddle dissolution method at 37° C.

Materials

All materials employed were of pharmaceutical grade.

Calcium hydrogen phosphate (Di-cafos A): Budenheim

Croscarmellose Sodium Ac-Di-Sol: FMC

Magnesium stearate: Magnesia GmbH

Polyethylene glycol: Hoechst

Lactose: DMV

Other materials employed appear from the following examples.

EXAMPLES

Example 1

Preparation of Tablets Containing a Particulate Material According to the Invention

The example illustrates the preparation of a particulate material comprising a relatively large amount of a carrier. The particulate material obtained exhibits good flowability, good compactability and possesses excellent tabletting properties. Thus, the particulate material allow the preparation of e.g. tablets and in spite of the relatively large load of carrier the tablets display minimal, if any, adherence (sticking) to tablet punches and/or dies during compression. Furthermore, the tablets obtained have acceptable properties with respect to disintegration, weight variation and hardness.

Starting Materials

Lactose monohydrate (DMV) 125 mesh

Calcium hydrogen phosphate anhydrous (Di-Ca-Fos P)

Polyethylene glycol 6000 (PEG 6000) having a melting point of about 60° C.

Equipment

Fluid bed Strea-1 (from Aeromatic-Fielder) mounted with a special developed top-spray binary nozzle having an opening of 0.8 mm.

Granular Compositions

Composition 1.1	
Lactose	500 g
PEG 6000	420 g (sprayed on lactose)

The composition has a carrier concentration of 45.6% w/w.

Composition 1.2	
Calcium hydrogen phosphate anhydrous	500 g
REG 6000	210 g (sprayed on calcium hydrogen phosphate)

The composition has a carrier concentration of 29.6% w/w.

Process Conditions—Description

Lactose (or for composition 1.2 calcium hydrogen phosphate anhydrous) was fluidised at appropriate inlet airflow. The inlet air was not heated. PEG 6000 was melted using an electrically heated pressure tank. The temperature was kept at a temperature at about 85° C., i.e. above the melting point of PEG 6000. The melt was pumped from the tank to the nozzle through a heated tube. In the tube, the temperature was kept at 80° C. The pressure in the tank determined the flow rate of the melt. The nozzle was heated to keep the droplets in a liquefied stage by means of heating the atomizer air delivered through the top-spray nozzle.

Settings

Inlet airflow: 30–50 m^3 per hour

Inlet air temperature: Ambient temperature (20–25° C.)

Tank temperature: 85° C.

Tank pressure: 1.5 Bar corresponding to a flow rate of 14–15 g/min

Tube temperature: 80° C.

Atomising air temperature: 100° C.

Process time: 28 min

Product temperature at equilibrium: 40° C. (after 15 minutes)

Product Characteristics

The products (composition 1.1 and 1.2) appear as free flowing granular products with a mean granule size of approx. 300–500 μm.

Tabletting

Compositions

Tablet formulation I (without disintegrant)	
Granular product	99% w/w
Magnesium stearate	1% w/w

The tablet formulation has a carrier concentration of 45.2% w/w.

Tablet formulation II (with disintegrant)	
Granular product	95% w/w
Ac-Di-Sol (croscarmelose sodium)	4% w/w (disintegrant)
Magnesium stearate	1% w/w

The tablet formulation has a carrier concentration of 28% w/w.

Tablet Properties

Tablet formulation I based on composition 1.1, i.e. with lactose

Tablet punch: Compound cup, 10 mm in diameter
Tablet machine: Single punch machine Korsch EK0
Tablet weight: 250 mg
Weight variation, RSD <1%
Average tablet hardness: 96 N
Average disintegration time: 10 min
Tablet appearance: White glossy tablets

Tablet formulation I based on composition 1.2, i.e. with dicalcium phosphate

Tablet punch: Compound cup, 10 mm in diameter
Tablet machine: Single punch machine Korsch EK0
Tablet weight: 450 mg
Weight variation, RSD <1%
Average tablet hardness: 121 N
Average disintegration time: 17 min
Tablet appearance: White glossy tablets

Tablet formulation II based on composition 1.1, i.e. with lactose

Tablet punch: Compound cup, 10 mm in diameter
Tablet machine: Single punch machine Korsch EK0
Tablet weight: 250 mg
Weight variation, RSD <1%
Average tablet hardness: 112 N
Average disintegration time: 8 min
Tablet appearance: White glossy tablets

Thus, addition of a disintegrant results in a decrease in the average disintegration time without any other changes of importance.

Tablet formulation II based on composition 1.2, i.e. with calcium hydrogen phosphate

Tablet punch: Compound cup, 10 mm in diameter
Tablet machine: Single punch machine Korsch EK0
Tablet weight: 450 mg
Weight variation, RSD <1%
Average tablet hardness: 118 N
Average disintegration time: 9 min
Tablet appearance: White glossy tablets

When calcium dihydrogen phosphate anhydrous is employed a more pronounced decrease in disintegration time is observed compared with that of lactose. The average tablet hardness is maintained at an excellent level.

Example 2

Controlled Agglomeration—Proof of Concept

Method

Controlled agglomeration is obtained by keeping the product temperature at minimum10° C. below melting point of the carrier reducing the probability of agglomeration due to coalescence. Controlled agglomeration is characterised by gradual increase in mean granule size (geometric weight mean diameter d_{gw}) as function of applied amount of carrier. In contrast, uncontrolled agglomeration shows rapidly increasing granule size. As a proof of concept the granule growth pattern are compared corresponding to the following conditions:

Inlet fluidising air temperature of ambient temperature: 20–25° C.

Inlet fluidising air temperature of 85° C. leading to a temperature of the product of about 50–60° C.,

Starting Materials

Lactose monohydrate 125 mesh
Polyethylene glycol 6000

Equipment

Fluid bed Strea-1 mounted with a top-spray binary nozzle.

Granular Compositions

Lactose	400 g
PEG 6000	Increased stepwise in separate experiments (from 0% to about 60% w/w in the final composition)

Process Conditions

The conditions were the same as described in Example 1.

Settings (Controlled Agglomeration)

Inlet airflow: 30–50 m^3 per hour
Inlet air temperature: Ambient temperature (20–25° C.)
Tank temperature: 90° C.
Tank pressure: 1.5 Bar corresponding to a flow rate of 14–15 g/min
Tube temperature: 85° C.
Atomizer air temperature: 100° C.
Product temperature at equilibrium: 40° C.

Settings (Uncontrolled Agglomeration)

Inlet airflow: 30–50 m^3 per hour
Inlet air temperature: 85° C.
Tank temperature: 90° C.
Tank pressure: 1.5 Bar corresponding to a flow rate of 14–15 g/min
Tube temperature: 85° C.
Atomizer air temperature: 100° C.
Product temperature at equilibrium: 55–65° C.

Product Characteristics

Increasing amounts of PEG were sprayed onto the fluidised lactose particles and the particle size distribution of the products was analysed by method of laser diffraction, dispersing the agglomerates in air. The correlation between mean granule size (geometric weight mean diameter d_{gw}) and applied amount of carrier demonstrates the difference between controlled and uncontrolled agglomeration as shown in FIG. 1 and Table 1. Table 1 includes the geometric standard deviation s_g related to the wideness of the size distribution.

TABLE 1

Particle size characteristics of granulate products produced by agglomeration by melt spraying in fluid bed at heated and unheated inlet air conditions at different applied amount of PEG 6000 concentrations.

Product temperature 40–45° C. Inlet air temperature: Ambient			Product temperature 50–60° C. Inlet air temperature: 85° C.		
PEG, w/w %	D_{gw}, µm	S_g	PEG w/w %	D_{gw}, µm	S_g
0	55	2.37	0	55	2.37
17	151	2.09	13	343	1.98
26	261	2.09	15	513	1.48
38	328	2.06	25	980	1.43
48	332	1.95			
60	450	1.8			

D_{gw}: Geometric weight mean diameter.
S_g: Geometric standard deviation.

Example 3

Improving tabletting Characteristics of Paracetamol Applying the controlled Agglomeration Technique

Parcetamol has been chosen as model substance representing a substance with poor compression characteristics. By incorporation of PEG 6000 by melt spraying, i.e. spraying melted PEG 6000 on paracetamol, a granular product of paracetamol is obtained with excellent flowability and tablet compression characteristics. In order to obtain tablets with satisfactory disintegration time Avicel PH 200 and Kollidon CL (super-disintegrant) has been added to the product

Starting Materials

Polyethyleneglycol 6000 (Hoechst)

Paracetamol (Unikem)

Equipment

Fluid bed Strea-1 (Aeromatic-Fielder)

Process Conditions

300 g PEG 6000 was melted by heating to 90° C. in a pressure tank. The melted carrier was pumped through a heated tube (85° C.) to the binary nozzle in the fluid bed at a tank pressure of 1.5 Bar. The atomizing air was heated to 140° C. The inlet air temperature of the fluid bed was 25° C.

241 g of PEG was sprayed on 250 fluidized paracetamol at a flow rate of 17 g/min. The total yield was 491 g granulate with a composition corresponding to 49,1% w/w PEG 6000 and 50.9% w/w paracetamol. The maximum product temperature was 36° C. at the end of the process.

Product Characteristics

The median particle size on volume basis is 85 µm for paracetamol was increased to 295 µm during the controlled agglomeration process. The median particle size was determined by laser diffraction (Helos) dispersing the particles in air.

Tablet composition	
Paracetamol	44%
PEG 6000	41%
Avicel PH200	10%

-continued

Tablet composition	
Kollidon CL	4%
Magnesium stearate	1%

Paracetamol and PEG 6000 are employed in the form of the granular product obtained as described above.

Avicel PH is blended with the granular product for 2 minutes in Turbula mixer and after adding magnesium stearate for further 0.5 minutes. Avicel PH200 (microcrystalline cellulose) is supplied by FMC, Kollidon CL by BASF and magnesium stearate by Magnesia GmbH.

Tabletting and Tablet Characteristics

The tabletting was performed on a single punch tabletting machine Korsch EK0

Tablet shape 8 mm doomed shape

Weight: 200 mg

Strength 87 mg

Mean tablet hardness (n=10) determined on a Schleuninger Model 6D apparatus was 77 N

Friability was 0.2% determined at a Roche friabililator

Mean disintegration time was 11 minutes (Ph.Eur)

Weight variation (n=20) corresponded to RSD of 0.6%

In conclusion, the tablets obtained from the granulate prepared by the controlled agglomeration method of the invention were very satisfactory and only a relatively small concentration of tabletting excipients was needed in order to ensure a suitable tabletting process. Furthermore, the example demonstrates that it is possible to obtain a granulate that has a relatively high concentration of carrier (about 50% w/w) and at the same time has a suitable particle size for further processing.

Example 4

In Vivo Bioavailability in Dogs After Administration of Tablets Containing a Particulate Material Obtained by the Controlled Agglomeration Method of the Present Invention—Proof of Concept

The present example illustrates that a composition containing a particulate material obtained according to the present invention leads to improved bioavailability after oral administration to dogs compared with compositions made by techniques that are generally accepted as useful when an increase in bioavailability is desired. In the present example compositions in the form of a nanosuspension and a microemulsion are used for comparison.

The model drug substance employed illustrates a drug substance that has a very low aqueous solubility of less than 50 ng/ml independent on pH. The molecular weight of the model drug substance is about 600 and it has a lipophilicity i.e. a log P (octanol/water) of 5.0.

Proof of concept is based on a comparison of bioavailability of different oral formulations and an I.V. injection of the drug substance in dogs (n=4). Data on the I.V. is not included in this example.

Treatment Compositions and Treatment Schedule

Treatment A (comparison treatment): nanosuspension containing 2% w/w of the model drug substance. NanoCrystal™ colloidal suspension of the model drug substance stabilised with hydroxy propyl cellulose (HPC-SL). Supplier: Élan pharmaceutical technologies, USA. EPT Ref.

NB: GOT-5747-170. The nanosuspension contains 2% of the model drug substance and 1% HPC-SL (w/w). A treatment consisted in oral administration of 36.3 mg as a sigle dose (approximately 1.8 ml).

Treatment B (according to the invention): tablets containing a particulate material obtained according to the method of the present invention. The tablets contain about 1% w/w of the model drug substance. The preparation of the composition used in Treatment B is described below. A treatment consisted in oral administration of 6 tablets as a single dose corresponding to approx. 37.5 mg.

Treatment C (according to the invention): tablets containing a particulate material obtained according to the method of the present invention. The tablets contain about 5% w/w of the model drug substance. The preparation of the composition used in Treatment C is described below. A treatment consisted in oral administration of 2 tablets as a single dose corresponding to approx. 42.4 mg.

Treatment D (comparison treatment): capsules containing a microemulsion of the model drug substance. Soft gelatine capsules containing 7.3 mg of the model drug substance in a vehicle consisting of 40% w/w Softigen 767, 15% w/w trietylcitrate and 45% w/w polysorbate 80 (0.05% BHA was added by weight as antioxidant). A treatment consisted of a single dose of 5 capsules, equivalent to 36.5 mg of the model drug substance.

Treatment E (comparison treatment): capsules containing a microemulsion of the model drug substance. Soft gelatine capsules containing 12.43 mg of the model drug substance in a vehicle consisting of 40% w/w Softigen 767, 15% w/w trietylcitrate and 45% w/w polysorbate 80 (0.05% BHA was added by weight as antioxidant). A treatment consisted of a single dose of 3 capsules, equivalent to 37.2 mg of the model drug substance.

Preparation of a Pharmaceutical Composition According to the Invention used in Treatment B (5 mg Model Drug Substance)

Preparation of a Particulate Material—Melt-spraying Process

Starting Materials

Polyethyleneglycol 6000 (Hoechst)

Poloxamer 188 (BASF)

Model drug substance

Avicel PH 101 (FMC)

Equipment

Fluid bed Strea-1 (Aeromatic-Fielder)

Process Conditions

198.0 g PEG 6000 and 85.0 g Poloxamer 188 (70:30 w/w) were melted by heating to 75° C. in a pressure tank. 6.21 g model drug substance was dissolved in the melted carriers. The melt was pumped through a heated tube (80° C.) to the binary nozzle in the fluid bed at a tank pressure of 1.8 Bar. The atomizing air was heated to 140° C. The inlet air temperature of the fluid bed was 22° C.

289 g of melt was sprayed on 300 g fluidized Avicel PH 101 at a flow rate of 10 g/min. The total yield was 589 g granulate. The maximum product temperature was 36° C. at the end of the process.

Product Characteristic

Granular, free flowing product with a particle size under 0.7 mm.

Tablet Composition (w/w)

Tablets were obtained by compression of a powder blend containing the granulate obtained as described above with magnesium stearate.

Model drug substance	1.04%
PEG 6000	33.26%
Poloxamer 188	14.29%
Avicel PH101	50.41%
Magnesium stearate	1.00%

Magnesium stearate was blended with the granulate for 0.5 minutes in a Turbula-mixer.

Tabletting and Tablet Characteristics

The tabletting was performed on a single punch tabletting machine Korsch EK0

Tablet shape 11.5 mm doomed shape

Weight: 515 mg

Strength 5 mg

Mean tablet hardness (n=10) determined on a Schleuninger Model 6D apparatus was 105 N

Mean disintegration time was 21.5 minutes (Ph.Eur)

Weight variation (n=20) corresponded to RSD of 0.9%

Preparation of a Pharmaceutical Composition According to the Invention used in Treatment C (20 mg Model Drug Substance)

Preparation of a Particulate Material—Melt-spraying Process

Starting Materials

Polyethyleneglycol 6000 (Hoechst)

Poloxamer 188 (BASF)

Model drug substance

Avicel PH 101 (FMC)

Equipment

Fluid bed Strea-1 (Aeromatic-Fielder)

Process Conditions

121.9 g PEG 6000 and 52.3 g Poloxamer 188 (70:30 w/w) were melted by heating to 75° C. in a pressure tank. 20.96 g model drug substance was dissolved in the melted carriers. The melt was pumped through a heated tube (80° C.) to the binary nozzle in the fluid bed at a tank pressure of 1.8 Bar. The atomizing air was heated to 140° C. The inlet air temperature of the fluid bed was 22° C.

195 g of melt was sprayed on 200 g fluidized Avicel PH 101 at a flow rate of 11.4 g/min. The total yield was 395 g granulate. The maximum product temperature was 37° C. at the end of the process.

Product Characteristic

Granular, free flowing product with a particle size under 0.7 mm.

Tablet Composition (w/w)

Tablets were obtained by compression of a powder blend containing the granulate obtained as described above with magnesium stearate.

Model drug substance	5.26%
PEG 6000	30.54%
Poloxamer 188	13.11%
Avicel PH101	50.09%
Magnesium stearate	1.00%

Magnesium stearate was blended with the granulate for 0.5 minutes in a Turbula-mixer.

Tabletting and Tablet Characteristics

The tabletting was performed on a single punch tabletting machine Korsch EK0

Tablet shape 11.5 mm doomed shape

Weight: 409 mg

Strength 20 mg

Mean tablet hardness (n=10) determined on Schleuninger Model 6D apparatus was 41 N

Mean disintegration time was 5.5 minutes (Ph.Eur)

Weight variation (n=20) corresponded to RSD of 1.3%

Study Design and Results

The study design was a cross-over study, which comprised all four dogs in one group. In each of totally six weeks the dogs were dosed orally on the first day of the week following by 6 days of recovery. The first week the dogs were assigned to treatment A, second week to treatment B etc.

Summary of pharmacokinetic parameters. Beagle dogs after single oral dosing of the model drug substance (±SD, n=4).

Treatment	A	B	C	D	E
t_{max} (h)	2.2 ± 0.5	2.8 ± 0.5	4.3 ± 3.2	2.8 ± 1.3	2.0 ± 0.0
C_{max} (ng/ml)	19 ± 8	52 ± 15	29 ± 17	35 ± 13	42 ± 6
$AUC_{0-inf}{}^{a}$ (ng/ml)	206 ± 108	489 ± 187	290 ± 184	318 ± 144	318 ± 65
F^{b} (%)	4.8 ± 1.9	11 ± 4	5.4 ± 2.7	7.8 ± 3.8	7.6 ± 2.9

Calculated as

$^{a}AUC_{last} + C_{last}{}^{*}t_{1/2,i.v.}/\ln 2$;

$^{b}AUC_{0-inf,po} \cdot D_{iv}/(AUC_{0-inf,iv} \cdot D_{po})$

From the results given above it and in FIG. 5 is seen that treatment B leads to improved bioavailability compared with all other treatments employed. It is particularly interesting to note that compositions containing the model drug substance in dissolved form (treatment D and E) do not lead to a better bioavailability than treatment B and there is no significant difference in the t_{max} values obtained, i.e. the onset of the therapeutic effect is the same even if a solid composition is used. Treatment C leads to a lower bioavailability than treatment B, which may be explained by the fact that the ration between the amount of drug substance in the carrier is higher in treatment C than in treatment B (higher dose in treatment C than in treatment B).

Example 5

In Vivo Bioavailability in Dogs After Administration of Tablets Containing a Particulate Material Obtained by the Controlled Agglomeration Method of the Present Invention—Proof of Concept II

The present example illustrates that a composition containing a particulate material obtained according to the present invention leads to improved bioavailability after oral administration to dogs compared with compositions made by techniques that are generally accepted as useful when an increase in bioavailability is desired. In the present example compositions in the form of a nanosuspension and a cyclodextrin solution are used for comparison.

The model drug substance employed illustrates a drug substance that has a very low aqueous solubility of about 50 pg/ml in phosphate buffer pH 7.4. The model drug substance in this example has a pK_A of 8, a molecular weight of about 450 and a lipophilicity i.e. a log P (octanol/buffer pH 7.4) of 6.0. The model drug substance is employed in the form of a hydrochloride salt. The aqueous solubility of the salt is also very low.

The results presented below are based on absorption study in dogs comparing 6 different formulations.

Formulation A (nanosuspension)

Formulation B: Cyclodextrin solution (Captisol)

Formulation C: Mixture of SLS and the model drug substance (0.5:1)

Formulation D: Mixture of SLS and the model drug substance (1:1)

Formulation E: Granulate with 10% Tween 80

Formulation F: (granulate in capsule) prepared by a method according to the present invention by melt spraying and using Akosoft XP 3103.

A summary of the pharmacokinetic report on the study is given below.

Test formulation A was prepared by suspending nanonised model drug substance particles in a vehicle of 0.5% HPC (HPC) (Klucel® MF EP, Hercules Inc.) and purified water. A similar suspension was included in an initial study where it resulted in a mean relative bioavailability of only 0.64 when compared to a 5% Captisol® solution. However, it was suspected that the initial suspension used was not optimal, as the particle size distribution was above the micrometer range. Subsequently, the micronisation process has been optimised, and test formulation A was prepared from a model drug substance batch, which contained particles in the nanometer range.

Reference formulation B was prepared by dissolving the model drug substance in an aqueous vehicle of 5% β-cyclodextrin sulfobutyl ether, sodium salt (Captisol®, CyDex Inc).

Test formulation C was prepared by dissolving sodium lauryl sulphate (SLS) in water and adding the solution to the model drug substance drop by drop (model drug substance/SLS w/w-ratio 2:1). The dried mixture and lactose were filled in capsules.

Test formulation D was prepared by dissolving SLS in water and adding the solution to the model drug substance drop by drop (model drug substance/SLS w/w-ratio 1:1). The dried mixture and lactose were filled in capsules.

Test formulation E was prepared by melt granulation of the model drug substance, 10% Tween 80, 2% Kollidon VA64, corn starch and lactose. The granulate was filled in capsules.

Test formulation F was prepared by a method of the invention by melt spraying the model drug substance, Akosoft 3103 and lactose. The granulate obtained was filled in capsules. Akosoft 3103 is a mixture of Akoline HH (C_8-C_{10} monoglycerides), Akosoft 36 (hydrogenated cocoglyceride) and Akofine NF (hydrogenated cottonseed oil) from Karlshamns AB. All are saturated fats or oils, i.e. no double-bonds, PEG-chains or free acid groups exist in the excipients.

In the following the preparation of test formulation F is described in further details.

Test Formulation F

Preparation of a Particulate Material—Melt-spraying Process

Starting Materials

Akosoft XP 3103 (Karlshamn)
Model drug substance
Lactose 350 M (DMV)

Equipment

Fluid bed Strea-1 (Aeromatic-Fielder)

Process Conditions

153 g Akosoft XP 3103 was melted by heating to 70° C. in a pressure tank. The melt was pumped through a heated tube (80° C.) to the binary nozzle in the fluid bed at a tank pressure of 0.3 Bar. The atomizing air was heated to 140° C. The inlet air temperature of the fluid bed was 22° C.

114 g of melt was sprayed on fluidized material consisting of 256.5 g lactose 350 M and 43.5 g model drug substance at a flow rate of 30 g/min. The total yield was 414 g granulate. The maximum product temperature was 32° C. at the end of the process.

Product Characteristic

Granular product with a particle size under 0.7 mm.

The product was filled into capsules (500 mg corresponding to 30 mg base)

Study Design and Dosing

The study was conducted in a cross-over design. After a five days pre-dose period the test formulations were administered in intervals of three or four days. Test formulations were administered in the order B, A, C, D, E and F.

On days of dosing each dog was dosed in the morning with 30 mg of the model drug substance (with regard to the base) irrespective of bodyweight. The dose level chosen was based on previous studies with the model drug substance in Beagle dogs.

Pharmacokinetic Results

Mean serum concentrations vs. time are presented in FIG. **6**. Standard deviations are omitted in the figure for clarity. The data is shown in the Table below

The concentration of the model drug substance in the serum sample taken from dog F1131 at 24 hours is high compared to the concentrations observed at previous time points. Re-analysis confirmed the result and the late serum concentration increase might therefore be due to delayed absorption of the test compound.

Pharmacokinetic parameters for the model drug substance estimated by standard non-compartmental analysis are given in the following Table.

For the reference solution a mean t_{max} of 2.5 hours was observed. The other treatments resulted in mean t_{max} values of 2.3 hours (HPC—formulation A), 3.0 hours (model drug substance/SLS 2:1—formulation C), 3.8 hours (Akosoft 3103—formulation F), 4.8 hours (Tween 80/Kollidon VA64—formulation E) and 8.3 hours (model drug substance/SLS 1:1—formulation D). The latter mean t_{max} value is high due to the extreme contribution from dog F1131 (see above). If this data point is omitted a mean t_{max} of 3.0 hours is observed.

With a mean maximum serum concentration at 123 nmol·L^{-1} the Akosoft 3103 formulation (formulaion F) gave a value almost similar to the reference solution at 124 nmol·L^{-1}. At the other extreme the treatments with SLS (formulations C and D) resulted in mean C_{max} values of 31.5 nmol·L^{-1} (model drug substance/SLS 2:1) and 50.3 nmol·L^{-1} (model drug substance/SLS 1:1). Again the mean value would be smaller if the 24 hours data point for formulation D was omitted. Administration of the HPC- and Tween 80/Kollidon VA64-formulations resulted in mean C_{max} values of 87.9 nmol·L^{-1} and 85.3 nmol·L^{-1}, respectively.

In the Table on next page are given individual and mean (n=4) pharmacokinetic parameters of the model drug substance employed in Example 5 after dosing of 30 mg to Beagle dogs. Treatment A: 0.5% HPC (aq.), Treatment B: 5% Captisol® (aq.), Treatment C: model drug substance/SLS (2:1), Treatment D: model drug substance/SLS (1:1), Treatment E: Tween 80, Kollidon VA64, corn starch and lactose, Treatment F: Akosoft® 3103.

Treatment[a)]	Animal	Dose[b)] (nmol/kg)	t_{max} (h)	C_{max} (nmol · L^{-1})	AUC_{0-t} (nmol · h · L^{-1})	AUC_{0-inf} (nmol · h · L^{-1})	$AUC_{\%residual}$	$t_{1/2}$[c)] (h)	CL/F (L · kg^{-1} · h^{-1})	V_2/F (L · kg^{-1})	$F_{rol,Inf}$[d)]	$F_{rol,t}$[e)]
A	F1131	4381	2.0	82.3	617	657	6.1	3.9	6.67	37.6	0.58	0.57
	F1132	4440	2.0	61.3	407	418	2.7	4.3	10.6	66.2	0.52	0.52
	F1138	4595	3.0	109	1025	1067	4.0	4.9	4.31	30.6	0.95	0.94
	F1139	5016	2.0	99.0	751	780	3.7	4.7	6.40	44.0	1.04	1.04
	Mean		2.3	87.9	700	731	4.1	4.5	7.0	44.6	0.77	0.77
	CV %		21.7	23.7	37.0	36.9	34.9	9.85	37.5	34.5	33.9	33.9
B	F1131	4730	3.0	145	1163	1231	5.5	5.3	3.84	29.6	—	—
(reference)	F1132	4794	3.0	101	842	873	3.5	4.7	5.49	37.2	—	—
	F1138	4995	2.0	141	1180	1217	3.0	4.7	4.11	27.6	—	—
	F1139	5560	2.0	107	804	832	3.4	4.7	6.71	45.4	—	—
	Mean		2.5	124	997	1038	3.9	4.9	5.0	35.0	—	—
	CV %		23	18	20	21	29	6.1	27	23	—	—

-continued

Treatment[a)]	Animal	Dose[b)] (nmol/kg)	t_{max} (h)	C_{max} (nmol · L^{-1})	AUC_{0-t} (nmol · h · L^{-1})	AUC_{0-inf} (nmol · h · L^{-1})	$AUC_{\%residual}$	$t_{1/2}$[c)] (h)	CL/F (L · kg^{-1} · h^{-1})	V_z/F (L · kg^{-1})	$F_{rel,Inf}$[d)]	$F_{rel,t}$[e)]
C	F1131	4762	3.0	12.5	78	95	17	4.1	50.2	297	0.08	0.07
	F1132	4794	3.0	8.63	53	66	20	4.3	73.0	455	0.08	0.06
	F1138	5030	3.0	6.86	51	73	30	6.1	69.3	608	0.06	0.04
	F1139	5580	3.0	98.0	781	817	4.4	4.9	8.83	48.2	0.98	0.97
	Mean		3.0	31.5	241	263	17.9	4.9	50	352.1	0.30	0.29
	CV %		0.0	141	150	141	58.9	18.4	61.0	67.9	151	158
D	F1131	4730	24	32.5	321	610	47	6.2	7.75	68.9	0.11	0.28
	F1132	4826	2.0	34.1	291	339	14	4.7	14.2	96.1	0.27	0.23
	F1138	4995	3.0	27.7	236	249	5.1	5.2	20.0	150	0.20	0.20
	F1139	5537	4.0	107	913	957	4.6	4.9	5.80	41.1	1.16	1.14
	Mean		8.3	50.3	440	539	17.7	5.3	12	89.0	0.44	0.46
	CV %		127	75.3	72.1	59.0	113	12.6	54.3	52.2	111	98.5
E	F1131	4826	6.0	43.2	575	752	24	10	8.42	95.4	0.60	0.48
	F1132	4859	6.0	78.8	802	835	4.0	4.5	5.82	38.1	0.94	0.94
	F1138	5030	3.0	102	956	1015	5.8	5.5	4.95	39.0	0.83	0.80
	F1139	5537	4.0	117	1058	1118	5.3	6.2	5.00	37.4	1.35	1.33
	Mean		4.8	85.3	848	930	9.8	6.3	5.5	52.5	0.93	0.89
	CV %		31.3	37.7	24.8	17.9	97.1	39.7	12.8	54.5	33.7	39.6
F	F1131	4762	3.0	152	1334	1414	5.7	5.3	3.37	25.6	1.14	1.14
	F1132	4826	4.0	99.1	839	867	3.3	4.4	5.56	35.1	0.99	0.99
	F1138	5102	4.0	88.1	881	926	4.8	5.0	5.51	40.1	0.74	0.73
	F1139	5537	4.0	153	1210	1266	4.4	4.8	4.37	30.1	1.53	1.52
	Mean		3.8	123	1066	1118	4.6	4.9	4.7	32.7	1.10	1.10
	CV %		13.2	27.9	22.9	23.6	21.6	7.70	22.2	19.2	30.0	31.0

Individual doses used in the pharmacokinetic analysis were calculated by $D \cdot CF/M_w \cdot BW$; D is the dose administered with respect to the base (ng), M_w is the molecular weight of the model drug substance (ng/nmol), BW is the body weight of the animal (kg) and CF is the correction factor determined from analysis of the test formulations.

$t_{1/2}$ was calculated from λ-values estimated from data points at 2–8 hours (I), 2–12 hours (II), 2–24 hours (III), 3–12 hours (IV), 3–24 hours (V), 4–24 hours (VI), 6 hours (VII) and 6–24 hours (VIII)

$F_{rel,\,inf}$ was calculated as $F_{rel}=AUC_{0-inf}^{test} \cdot Dose^{ref}/AUC_{0-inf}^{ref} \cdot Dose^{test}$

$F_{rel,\,t}$ was calculated as $F_{rel}=AUC_{0-t}^{test} \cdot Dose^{ref}/AUC_{0-t}^{ref} \cdot Dose^{test}$

Mean values for the relative bioavailability (relative to cyclodextrin solution) were almost identical irrespective of the calculation being made with respect to the serum concentration time curve to infinity (AUC_{0-inf}) or to the last measurable concentration (AUC_{0-t}). Mean values for the latter AUC parameter were 997 $nmol \cdot h \cdot L^{-1}$ (reference formulation), 1066 $nmol \cdot h \cdot L^{-1}$ (Akosoft 3103), 848 $nmol \cdot h \cdot L^{-1}$ (Tween 80/Kollidon VA64), 700 $nmol \cdot h \cdot L^{-1}$ (HPC) 440 $nmol.h.L^{-1}$ (model drug substance/SLS 1:1) and 241 $nmol.h.L^{-1}$ (model drug substance/SLS 2:1). The low values for the two SLS formulations are in line with the low C_{max} values observed for these formulations.

The corresponding mean relative bioavailability-values were 1.10 (Akosoft 3103), 0.89 (Tween 80/Kollidon VA64), 0.77 (HPC), 0.46 (model drug substance/SLS 1:1) and 0.29 (model drug substance/SLS 2:1).

The low relative bioavailability observed for the two SLS-formulations was not expected as a similar formulation, albeit with a model drug substance/SLS-ratio at 2:1, administered in a previous study resulted in a mean relative bioavailability of 1.20. Apparently there is a critical concentration below which the dissolution- and absorption enhancing properties of SLS are limited.

All formulations administered to animal F 1039 resulted in a relative bioavailability (based on AUC_{0-inf}) around or above unity (range 0.98–1.53). The relative bioavailability determined in this dog for the different formulations therefore contributes considerably to the mean F_{rel}. This is especially the case for the two SLS formulations where the relative bioavailability is very low for the other three dogs. When this dog was excluded mean values of 0.24 and 0.06 were found for model drug substance/SLS-ratios of 1:1 and 2:1, respectively.

The mean apparent half life determined after administration of the various treatments were 4.5 hours (HPC suspension), 4.8 hours (5% Captisol® and model drug substance/SLS 2:1), 4.9 hours (Akosoft 3103), 5.2 hours (model drug substance/SLS 1:1) and 6.4 hours (Tween 80/Kollidon VA64). Mean oral clearances (CL/F) were comparable for treatments with HPC (7.01 $L \cdot kg^{-1} \cdot h^{-1}$), 5% Captisol® (5.04 $L \cdot kg^{-1} \cdot h^{-1}$), Tween 80/Kollidon VA64 (5.54 $L \cdot kg^{-1} \cdot h^{-1}$) and Akosoft 3103 (4.70 $L \cdot kg^{-1} \cdot h^{-1}$). As a consequence of the low AUC_{0-inf} values the two treatments with SLS show relatively high CL/F values at 12 $L \cdot k^{-1} \cdot h^{-1}$ (model drug substance/SLS 1:1) and 50 $L \cdot {}^{-1} \cdot h^{-1}$ (model drug substance/SLS 2:1).

Mean volumes of distribution (V_z/F) observed were 29.6 $L \cdot k^{-1}$ (HPC), 32.7 $L \cdot k^{-1}$ (Akosoft 3103), 34.9 $L \cdot {}^{-1}$ (5% Captsol®) and 52.5 $L \cdot {}^{-1}$ (Tween 80/Kollidon VA64). Again the values for the two SLS formulations were relatively higher at 158 $L \cdot k^{-1}$ and 352 $L \cdot k^{-1}$.

Pharmacokinetic parameters estimated for the reference solution were consistent with values found in a previous formulation study performed on identical animals.

As supplement to these data other formulations have been prepared including Captisol formulations: B (similar to the one in the previous study), and three formulations prepared according to the invention, formulation G, H and I. These formulations include mixtures of glycerides. Formulations G, H and I (granulate in capsule) have been manufactured by melt spraying.

Preparation of Test Formulation G, H and I According to the Invention

Preparation of a Particulate Material—Melt-spraying Process

Starting Materials

Kimol C 8–50 (Mono-diglycerid on medium chain fatty acids) (Cognis)

Viscoleo (medium chain triglycerides) (Grünau Illertissen)

Rylo MG 18 Pharma (Danisco Cultor)

Sodium lauryl sulfate (Millchem Limited)

Ascorbyl palmitate (Merck)

Model drug substance (the same substance is used throughout Example 5)

Lactose 350 M (DMV)

Equipment

Fluid bed Strea-1 (Aeromatic-Fielder)

Compositions			
Material	Formulation G g	Formulation H g	Formulation I g
Rylo MG 18	25.8	25.8	25.8
Viscoleo	21.2	21.2	21.2
Kimol	21.2	21.2	21.2
Model drug	50.2	50.2	25.1
Lactose 350 M	202.1	248.1	275.7
SLS	46.0	—	—
Ascorbyl palmitate	1.8	1.8	1.8

Process Conditions

The process conditions are similar for the formulation G, H and I. Rylo MG 18 was melted by heating to 70° C. in a pressure tank and the liquids Viscoleo and Kimol were added. The melt was pumped through a heated tube (80° C.) to the binary nose in the fluid bed at a tank pressure of 0.2 Bar. The atomizing air was heated to 140° C. The inlet air temperature of the fluid bed was 22° C.

The melt was sprayed on fluidized material consisting of the particulate materials, which include the model drug substance, lactose and ascorbyl palmitate and for formulation G; sodium lauryl sulfate. The flow rate was 20–30 g/min. The maximum product temperature was 32° C. at the end of the process.

Product Characteristic

Granular product with a particle size under 0.7 mm.

The product was filled into capsules (250 mg corresponding to 30 mg base for Formulation G and H). 500 mg corresponding to 30 mg base for formulation I.

Example 6

Proof of Concept Based on Data From Development Project with Nifedipine

Nifedipine is a yellow crystalline substance, practically insoluble in water with a solubility of <56 mg/L at 25° C. It has a molecular weight of 346.3 and a melting range between 172–174° C. The calculated log P is 2.5 and the experimental measured value is 2.2. Nifedipine is rapidly and fully absorbed after oral administration of the marketed products, however an immediate release capsule only produce a bioavailability between 30 and 60%.

Proof of concept is based on a comparison of bioavailability of different oral formulations with a solution of the drug substance as reference, in dogs in a cross over design. A summary is given below including detailed information on the melt spraying process and tabletting (Treatment B and C)

Treatment A

Solution of nifedipine in PEG 400

Composition	
Nifedipine	2% w/w
PEG 400	98% w/w

1 ml per capsule (corresponds to 20 mg nifedipine)

Treatment B

Plain tablet 20 mg Adalat® Bayer

Treatment C

Tablets prepared from a particulate material produced according to the present invention by melt spraying. Nifedipine is contemplated to be present in PEG/poloxamer as a solid solution.

Melt-spraying Process

Starting Materials

Polyethyleneglycol 6000 (Hoechst)

Poloxamer 188 (BASF)

Nifedipine (Sigma-Aldrich)

Lactose 200 mesh (DMV)

Equipment

Fluid bed Strea-1 (Aeromatic-Fielder)

Process Conditions

264.6 g PEG 6000 and 113.4 g Poloxamer 188 (70:30 w/w) were melted by heating to 90° C. in a pressure tank. 15.27 g drug substance was dissolved in the melted carriers. The melt was pumped through a heated tube (85° C.) to the binary nozzle in the fluid bed at a tank pressure of 1.6 Bar. The atomizing air was heated to 140° C. The inlet air temperature of the fluid bed was 22° C.

308 g of melt was sprayed on 300 g fluidized lactose at a flow rate of 17 g/min. The total yield was 608 g granulate. The maximum product temperature was 37° C. at the end of the process.

Product Characteristic

Granular, free flowing product with a particle size under 0.7 mm

Tablet composition	
Nifedipine	1.94% w/w
PEG 6000	33.71% w/w
Poloxamer 188	14.45% w/w
Avicel PH101	48.90% w/w
Magnesium stearate	1.00% w/w

Magnesium stearate was blended with the granulate for 0.5 minutes in a Turbula-mixer.

Tabletting and Tablet Characteristics

The tabletting was performed on a single punch tabletting machine Korsch EK0

Tablet shape 8 mm compound shape

Weight: 260 mg

Strength 5 mg

Mean tablet hardness (n=10) determined on a Schleuniger model 6D was 97 N

Mean disintegration time was 11.3 minutes (Ph.Eur)

Weight variation (n=20) corresponded to RSD of 1.15%

Dosing 4 tablets (20 mg) in a capsule

Dosing

One dog was dosed with the 3 different formulations A, B and C with 3 days between dosing. 2 ml of blood samples were taken at pre-dose and 0.25, 0.5, 1, 1.5, 2, 4, 8 and 24 hours after administration. The analysis of nifedipine was performed on respective plasma samples.

Pharmacokinetic Results

The pharmacokinetic data are shown in the Table below

Formulation	A	B	C
T_{max} (h)	0.5	0.5	1.0
C_{max} (ng/ml)	66.6	22.0	61.0
$AUC_{0-inf}{}^{a}$ (ng h/ml)	172.2	22.2	53.1
$F_{rel}{}^{b}$ (%)	100	12.9	30.8

Calculated as
$^{a}AUC_{last} + C_{last}/\lambda_z$;
$^{b}AUC_{0-inf,po} * D_{ref}/(AUC_{0-inf,ref} * D_{po})$

The bioavailability F_{rel} is calculated relative to formulation A, representing a solution of nifedipine in PEG 400. The corresponding plasma profiles are shown in FIG. 7.

Conclusion

Apparently the solid solution of nifedipine in PEG6000/Poloxamer (formulation C) results in significant higher bioavailability compared to a plain tablet formulation (Adalat).

Example 7

Neusilin as Absorption Material in Controlled Agglomeration

Background

It is established that magnesium aluminium silicate (Carrisorb, Gelsorp, Magnabite) is suitable in absorption of liquids and commonly used as a viscosity increasing, a tablet disintegrant and a tablet binding agent.

Neusilin (Fuji Chemical Industries) is a magnesium aluminometasilicate based on a polymeric reaction of sodium silicate having a siloxane structure (U.S. Pat. No. 3,959,444) in combination with a mixture or sodium aluminate and magnesium salts.

Neusilin US2 is a spray dried free flowing material with a particle size of approx. 80 μm and a specific surface area of 300 m^2/g.

Two experiments (A and B) have been performed where PEG 6000 is sprayed on fluidized Neusilin in a fluid bed Strea-1.

Experiment A is performed under conditions of controlled agglomeration keeping the temperature difference over 10° C. between the product and the melting point of PEG 6000 (59° C.).

Experiment B is performed under heating condition of the inlet air (50–70° C.) resulting in a product temperature under the 10° C. temperature difference.

Experiment A

Equipment

Fluid bed Strea-1 (Aeromatic-Fielder)

Process Conditions

1000 g PEG 6000 was melted by heating to 90° C. in a pressure tank. The melt was pumped through a heated tube (85° C.) to the binary nozzle in the fluid bed at a tank pressure of 1.5 Bar. The atomizing air was heated to 140° C. The inlet air temperature of the fluid bed was 22° C.

584 g of melt was sprayed on 150 g fluidized Neusilin US2 at a flow rate of 19 g/min. The total yield was 734 g granulate. The maximum product temperature was 45° C. at the end of the process. The concentration of PEG 6000 in the particulate material obtained was 79.6% w/w.

Product Characteristic

Granular, free flowing product with a particle size d_{gw} of 409 μm.

Tablet composition	
PEG 6000	79.6%
Neusilin	20.4%

Tabletting and Tablet Characteristics

The tabletting was performed on a single punch tabletting machine Korsch EK0. It was not necessary to add further excipients for the tabletting procedure.

Tablet shape 8 mm compound cup

Weight: 200 mg

Mean tablet hardness (n=10) determined on a Schleuniger model 6D was 48.6 N

Mean disintegration time was 22.4 minutes (Ph.Eur)

Weight variation (n=20) corresponded to RSD of 0.6%

Experiment B

Equipment

Fluid bed Strea-1 (Aeromatic-Fielder)

Process Conditions 800 g PEG 6000 was melted by heating to 90° C. in a pressure tank. The melt was pumped through a heated tube (85° C.) to the binary nozzle in the fluid bed at a tank pressure of 1.5 Bar. The atomizing air was heated to 140° C. The inlet air temperature of the fluid bed was 60° C.

505 g of melt was sprayed on 150 g fluidized Neusilin US2 at a flow rate of 19 g/min. The total yield was 655 g granulate. The maximum product temperature was 58° C. at the end of the process.

Product Characteristic

Granular, free flowing product with a particle size under 0.7 mm.

Tablet composition	
PEG 6000	77.1%
Neusilin	22.9%

Tabletting and Tablet Characteristics

Tabletting was not possible due to adhesion to the punches.

Conclusion

Neusilin US2 acts as an absorption agent for the melted carrier sprayed on the fluidized material.

Surprisingly high amount of carrier was applicable corresponding to a total amount of carrier exceeding 80% without getting uncontrolled agglomeration. In Experiment A, the temperature difference between product and melting point of the carrier exceeded 10° C. Further, direct tabletting of the product without adding lubricant was successfully performed.

Increasing the inlet temperature of the fluidized bed (Experiment B) exceeding the temperature limits for controlled agglomeration (recognized for the traditionally employed excipients) did not result in uncontrolled agglomeration as expected. This is most likely due to the high absorption capacity of Neusilin preventing free surface liquid to form bondings between the fluidized particles. However, uncontrolled agglomeration occurred at the end of the process (77.1% PEG 6000). Direct compression of the product was not possible due to adhesion to the punches indicating surface free PEG in the agglomerates, which might be due to the elevated product temperature in the agglomeration process.

To sum up, it is possible to obtain controlled agglomeration even in those cases where no or only a small temperature difference is present between the carrier and the second composition. This applies especially for substances like Neusilin and the like.

Example 8

Lubricant Effect of Neusilin in Comparison with Magnesium Stearate and Aerosil 200

A sticky granulate was produced by controlled agglomeration. PEG 1500 (melting range of from about 44 to about 48° C.) was applied on lactose 200 mesh in a fluid bed Strea-1. The composition of the product was as follows:

Lactose 200 mesh	300 g
PEG 1500	200 g

The granulate was sieved through a 0.71 mm mesh size.

A part of the granulate was blended with the different substances for 3 minutes in a Turbula mixer in order to determine any lubricating effect. Two of the substances used, namely magnesium stearate and Aerosil, are known lubricants. The substances employed were:

Neusilin ULF2 (Fuji Chemical Industries)

Magnesium stearate (Magnesia GmbH)

Aerosil 200 (colloidal silicon dioxide), (Degussa AG)

Tablets were produced on a single punch tabletting machine Korsch EK0, instrumented with force transducer on the filling device measuring the force to push off the tablet from the lower punch.

Tablet diameter 8 mm. Tablet shape: Compound cup

Tablet weight: 200 mg

The results are summarised in the Table below

Lubricant	Conc. %	Adhesion to tablet punches	Mean Push off force N
Neusilin	2	no	4.5
	4	no	1.1
Mg-stearate	1	Adhesion	n.m.
Aerosil 200	0.5	Adhesion	n.m
	1	Adhesion	n.m

Conclusion

Neusilin and Aerosil provided excellent flowability to the sticky granular product, whereas magnesium stearate did not have this effect. Aerosil is normally used as lubricant in the concentrations below 0.5% and is primarily used to improve the flowability of cohesive materials.

The anti-adhesive property of Neusilin is superior to both magnesium stearate and Aerosil. Granules blended with either 2 or 4% of Neusilin was compressed without any adhesion to the punches. As shown in the Table the adhesion to the lower punch was significantly decreased when increasing the concentration of Neusilin from 2 to 4%. The push off force was not monitored (n.m.) for the other lubricants since compression of tablets was not possible due to immediately adhesion to the punches.

Thus, the results demonstrate that Neusilin is an excellent lubricant having anti-adhesive properties.

The invention claimed is:

1. A method for preparing particulate material, comprising:

i) spraying a first composition on a second composition, wherein the first composition comprises one or more therapeutically or prophylactically active substances and a carrier in liquid form, wherein the carrier has a melting point of at least about 5° C., and the second composition comprises a material in solid form at a temperature corresponding to or below the melting point of the first composition, and

ii) agglomerating the first composition with the second composition to obtain the particulate material, wherein the therapeutically or prophylactically active substance has an aqueous solubility of at most about 3 mg/ml at 25° C. and a pH of about 7.4 and wherein the particulate material obtained comprises a geometric weight mean diameter from between about 75 to about 2000 μm.

2. The method of claim **1**, wherein the therapeutically active or prophylactic substance has an aqueous solubility of at most about 1 mg/ml at about 25° C. and a pH of about 7.4.

3. The method of claim **1**, wherein the therapeutically or prophylactically active substance has an aqueous solubility of at most about 0.01 mg/ml at about 25° C. and a pH of about 7.4.

4. The method of claim **1**, wherein the carrier has a melting point of about 10° C. or more.

5. The method of claim **1**, wherein the carrier has a melting point of at least about 20° C.

6. The method of claim **1**, wherein the carrier has a melting point of at least about 25° C.

7. The method of claim **1**, wherein the temperature of the second composition is at least about 2° C. below the melting point temperature of the carrier or the first composition.

8. The method of claim 1, wherein the temperature of the second composition is at least about 5° C. below the melting point temperature of the carrier or the first composition.

9. The method of claim 1, wherein the temperature of the second composition is at least about 10° C. below the melting point temperature of the carrier or the first composition.

10. The method of claim 1, which agglomeration is carried out in a high shear mixer, a low shear mixer, or a fluid bed.

11. The method of claim 1, which agglomeration is carried out in a fluid bed and wherein the first composition is sprayed on the second composition in a fluidized state.

12. The method of claim 1, wherein the spraying is performed through a spraying device equipped with temperature controlling means.

13. The method of claim 1, wherein the particulate material has a geometric weight mean diameter d_{gw} of at least about 10 micrometer.

14. The method of claim 1, wherein the particulate material has a geometric weight mean diameter d_{gw} of between about 20 micrometer and about 2000 micrometer.

15. The method of claim 1, wherein the concentration of the carrier in the particles is from about 5 to about 95% v/v.

16. The method of claim 1, wherein the first composition is liquefied by heating the carrier or the first composition to a temperature, which causes the carrier or the carrier composition to melt.

17. The method of claim 16, wherein the liquefied carrier or carrier composition has a viscosity (Brookfield DV-III) of at most about 800 mPas at a temperature of at most about 100° C.

18. The method of claim 1, wherein the first composition is essentially non-aqueous and contains at most about 20% w/w water.

19. The method of claim 1, wherein the carrier has a melting point of at most about 300° C.

20. The method of claim 1, wherein the carrier is a hydrophilic carrier, a hydrophobic carrier, a surfactant or a mixture thereof.

21. The method of claim 20, wherein the carrier is selected from one or more of polyether glycols; polyoxyethylenes, polyoxypropylenes; poloxamers and mixtures thereof.

22. The method of claim 20, wherein the carrier is selected from one or more of polyethylene glycol and polypropylene glycol.

23. The method of claim 20, wherein the carrier is selected from one or more of xylitol, sorbitol, potassium sodium tartrate, sucrose tribehenate, glucose, rhamnose, lactitol, behenic acid, hydroquinon monomethyl ether, sodium acetate, ethyl fumarate, myristic acid, citric acid; polyglycolized glycerides Gelucire 50/13, Gelucire 44114, Gelucire 50/10, Gelucire 62/05; Sucro-ester 7, Sucro-ester 11, Sucro-ester 15, maltose, mannitol and mixtures thereof.

24. The method of claim 20, wherein the carrier is selected from one or more of straight chain saturated hydrocarbons, sorbitan esters paraffins; fats and oils, cacao butter, beef tallow, lard, polyether glycol esters; higher fatty acids, stearic acid, myristic acid, palmitic acid, higher alcohols, cetanol, stearyl alcohol, low melting point waxes, glyceryl monostearate, hydrogenated tallow, myristyl alcohol, stearyl alcohol, substituted and/or unsubstituted monoglycerides, substituted and/or unsubstituted diglycerides, substituted and/or unsubstituted triglycerides, yellow beeswax, white beeswax, camauba wax, castor wax, japan wax, acetylate monoglycerides; NVP polymers, PVP polymers, and acrylic polymers.

25. The method of claim 1, wherein the carrier is polyethylene glycol having an average molecular weight from between about 400 to about 35,000.

26. The method of claim 1, wherein the carrier is selected from the group consisting of polyethylene glycol 1,000, polyethylene glycol 2,000, polyethylene glycol 3,000, polyethylene glycol 4,000, polyethylene glycol 5,000, polyethylene glycol 6,000, polyethylene glycol 7,000, polyethylene glycol 8,000, polyethylene glycol 9,000 polyethylene glycol 10,000, polyethylene glycol 15,000, polyethylene glycol 20,000, and polyethylene glycol 35,000.

27. The method of claim 1, wherein the carrier is polyethylene oxide having a molecular weight of from between about 2,000 to about 7,000,000.

28. The method of claim 1, wherein the carrier is a poloxamer.

29. The method of claim 1, wherein the carrier is selected from the group, consisting of Poloxamer 188, Poloxamer 237, Poloxamer 338 and Poloxamer 407.

30. The method of claim 1, wherein the carrier is selected from the group consisting of sorbitan esters, sorbitan. diisostearate, sorbitan dioleate, sorbitan monolaurate, sorbitan monoisostearate, sorbitan monooleate, sorbitan monopalmitate, sorbitan monostearate, sorbitan sesqui-isostearate, sorbitan sesquloleate, sorbitan sesquistearate, sorbitan tri-isostearate, sorbitan trioleate, sorbitan tristearate and mixtures thereof.

31. The method of claim 1, wherein the first composition comprises a mixture of one or more of hydrophilic and hydrophobic carriers.

32. The method of claim 1, wherein the second composition comprises one or more therapeutically active or prophylactic substances.

33. The method of claim 1, wherein the first composition further comprises one or more pharmaceutically acceptable excipients.

34. The method of claim 33, wherein the pharmaceutically acceptable excipient is selected from the group consisting of fillers, binders, disintegrants, glidants, coloring agents, taste-masking agents, pH-adjusting agents, solubilizing agents, stabilizsing agents, wetting agents, surface active agents, and antioxidants.

35. The method of claim 1, wherein the second composition comprises one or more pharmaceutically acceptable excipients.

36. The method of claim 35, wherein the pharmaceutically acceptable excipient is one or more of fillers, binders, disintegrants, glidants, coloring agents, taste-masking agents, pH-adjusting agents, solubilizing agents, stabilizing agents, wetting agents, surface active agents, and antioxidants.

37. The method of claim 1, wherein the first or the second composition comprises a cosmetically active substance, a beneficial substance, a food substance, or a nutrient substance.

38. The method of claim 1, wherein the second composition comprises magnesium aluminosilicate or magnesium aluminometasilicate and the amount of carrier in the particulate material is at least about 30% v/v.

39. The method of claim 38, wherein the amount of carrier in the particulate material is at least about 40% v/v.

40. The method of claim 38, wherein the amount of carrier in the particulate material is at least about 50% v/v.

41. The method of claim 1, wherein the particulate material is suitable for use in the preparation of pharmaceutical, cosmetic or food composition in a liquid, semi-solid or solid form.

42. The method of claim 1, wherein the particulate material is suitable for use in the preparation of tablets.

43. A method for improving the shelf-life of a pharmaceutical composition comprising an oxidation-sensitive therapeutically or prophylactically active substance, comprising subjecting the substance, before or during manufacture of the pharmaceutical composition, to the method of claim 1 by incorporating the substance in the first composition.

44. A method for preparing particulate material comprising spraying, on a second composition in a fluidized state, an amount of a first composition effective for agglomerating the second composition to obtain the particulate material, wherein the first composition comprises one or more therapeutically or prophylactically active substances having an aqueous solubility of at most about 3 mg/ml at 25° C. and a pH of about 7.4 and a carrier in liquid form, wherein the carrier has a melting point of at least about 5° C., the second composition comprises a material in solid form at a temperature corresponding to or below the melting point of the first composition, and the particulate material obtained has a geometric weight mean diameter from between about 75 to about 2000 μm.

* * * * *

EXHIBIT 6

Orange Book: Approved Drug Products with Therapeutic Equivalence Evaluations

Active Ingredient Search Results from "OB_Rx" table for query on "meloxicam."

Appl No	TE Code	RLD	Active Ingredient	Dosage Form; Route	Strength	Proprietary Name	Applicant
N021530		Yes	MELOXICAM	SUSPENSION; ORAL	7.5MG/5ML	MOBIC	BOEHRINGER INGELHEIM
A077882	**AB**	No	MELOXICAM	TABLET; ORAL	15MG	MELOXICAM	APOTEX INC
A077882	**AB**	No	MELOXICAM	TABLET; ORAL	7.5MG	MELOXICAM	APOTEX INC
A078008	**AB**	No	MELOXICAM	TABLET; ORAL	15MG	MELOXICAM	AUROBINDO PHARMA
A078008	**AB**	No	MELOXICAM	TABLET; ORAL	7.5MG	MELOXICAM	AUROBINDO PHARMA
A078039	**AB**	No	MELOXICAM	TABLET; ORAL	15MG	MELOXICAM	BEIJING DOUBLE CRANE
A078039	**AB**	No	MELOXICAM	TABLET; ORAL	7.5MG	MELOXICAM	BEIJING DOUBLE CRANE
N020938	**AB**	Yes	MELOXICAM	TABLET; ORAL	15MG	MOBIC	BOEHRINGER INGELHEIM
N020938	**AB**	No	MELOXICAM	TABLET; ORAL	7.5MG	MOBIC	BOEHRINGER INGELHEIM
A077920	**AB**	No	MELOXICAM	TABLET; ORAL	15MG	MELOXICAM	BRECKENRIDGE PHARM
A077920	**AB**	No	MELOXICAM	TABLET; ORAL	7.5MG	MELOXICAM	BRECKENRIDGE PHARM

A077937	**AB**	No	MELOXICAM	TABLET; ORAL	15MG	MELOXICAM	CARACO
A077937	**AB**	No	MELOXICAM	TABLET; ORAL	7.5MG	MELOXICAM	CARACO
A077918	**AB**	No	MELOXICAM	TABLET; ORAL	15MG	MELOXICAM	CARLSBAD
A077918	**AB**	No	MELOXICAM	TABLET; ORAL	7.5MG	MELOXICAM	CARLSBAD
A077930	**AB**	No	MELOXICAM	TABLET; ORAL	15MG	MELOXICAM	COREPHARMA
A077930	**AB**	No	MELOXICAM	TABLET; ORAL	7.5MG	MELOXICAM	COREPHARMA
A077931	**AB**	No	MELOXICAM	TABLET; ORAL	15MG	MELOXICAM	DR REDDYS LABS INC
A077931	**AB**	No	MELOXICAM	TABLET; ORAL	7.5MG	MELOXICAM	DR REDDYS LABS INC
A077932	**AB**	No	MELOXICAM	TABLET; ORAL	15MG	MELOXICAM	GLENMARK GENERICS
A077932	**AB**	No	MELOXICAM	TABLET; ORAL	7.5MG	MELOXICAM	GLENMARK GENERICS
A077944	**AB**	No	MELOXICAM	TABLET; ORAL	15MG	MELOXICAM	LUPIN PHARMS
A077944	**AB**	No	MELOXICAM	TABLET; ORAL	7.5MG	MELOXICAM	LUPIN PHARMS
A077934	**AB**	No	MELOXICAM	TABLET; ORAL	15MG	MELOXICAM	MYLAN
A077923	**AB**	No	MELOXICAM	TABLET; ORAL	15MG	MELOXICAM	MYLAN
A077934	**AB**	No	MELOXICAM	TABLET; ORAL	7.5MG	MELOXICAM	MYLAN
A077923	**AB**	No	MELOXICAM	TABLET; ORAL	7.5MG	MELOXICAM	MYLAN
A077938	**AB**	No	MELOXICAM	TABLET; ORAL	15MG	MELOXICAM	PURACAP PHARM
A077938	**AB**	No	MELOXICAM	TABLET; ORAL	7.5MG	MELOXICAM	PURACAP PHARM

A077928	**AB**	No	MELOXICAM	TABLET; ORAL	15MG	MELOXICAM	STRIDES ARCOLAB LTD
A077928	**AB**	No	MELOXICAM	TABLET; ORAL	7.5MG	MELOXICAM	STRIDES ARCOLAB LTD
A078102	**AB**	No	MELOXICAM	TABLET; ORAL	15MG	MELOXICAM	TARO
A078102	**AB**	No	MELOXICAM	TABLET; ORAL	7.5MG	MELOXICAM	TARO
A077936	**AB**	No	MELOXICAM	TABLET; ORAL	15MG	MELOXICAM	TEVA PHARMS
A077936	**AB**	No	MELOXICAM	TABLET; ORAL	7.5MG	MELOXICAM	TEVA PHARMS
A077927	**AB**	No	MELOXICAM	TABLET; ORAL	15MG	MELOXICAM	UNICHEM
A077927	**AB**	No	MELOXICAM	TABLET; ORAL	7.5MG	MELOXICAM	UNICHEM
A077929	**AB**	No	MELOXICAM	TABLET; ORAL	15MG	MELOXICAM	WATSON LABS
A077929	**AB**	No	MELOXICAM	TABLET; ORAL	7.5MG	MELOXICAM	WATSON LABS
A077921	**AB**	No	MELOXICAM	TABLET; ORAL	15MG	MELOXICAM	ZYDUS PHARMS USA
A077921	**AB**	No	MELOXICAM	TABLET; ORAL	7.5MG	MELOXICAM	ZYDUS PHARMS USA

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FDA/Center for Drug Evaluation and Research
Office of Generic Drugs
Division of Labeling and Program Support
Update Frequency:
Orange Book Data - **Monthly**
Generic Drug Product Information & Patent Information - **Daily**
Orange Book Data Updated Through December, 2011
Patent and Generic Drug Product Data Last Updated: February 10, 2012

EXHIBIT 7

ATTENTION DISPENSER: Accompanying Medication Guide must be dispensed with this product.

Mobic®
(meloxicam)
Tablets 7.5 mg and 15 mg
and
Mobic®
(meloxicam)
Oral Suspension 7.5 mg/5 mL

Boehringer Ingelheim

Rx only

Prescribing Information

WARNING

Cardiovascular Risk

- **NSAIDs may cause an increased risk of serious cardiovascular thrombotic events, myocardial infarction, and stroke, which can be fatal. This risk may increase with duration of use. Patients with cardiovascular disease or risk factors for cardiovascular disease may be at greater risk (see WARNINGS and CLINICAL TRIALS).**

- **MOBIC tablets/oral suspension is contraindicated for the treatment of peri-operative pain in the setting of coronary artery bypass graft (CABG) surgery (see WARNINGS).**

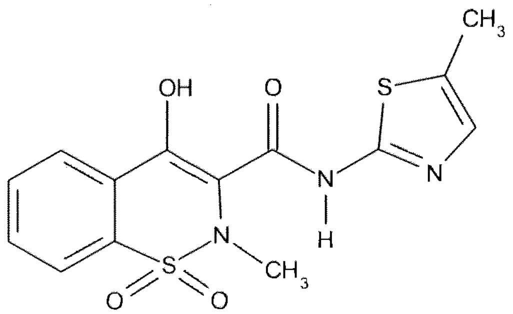
Gastrointestinal Risk

- **NSAIDs cause an increased risk of serious gastrointestinal adverse events including bleeding, ulceration, and perforation of the stomach or intestines, which can be fatal. These events can occur at any time during use and without warning symptoms. Elderly patients are at greater risk for serious gastrointestinal events (see WARNINGS).**

DESCRIPTION

Meloxicam, an oxicam derivative, is a member of the enolic acid group of nonsteroidal anti-inflammatory drugs (NSAIDs). Each pastel yellow MOBIC tablet contains 7.5 mg or 15 mg meloxicam for oral administration. Each bottle of MOBIC oral suspension contains 7.5 mg meloxicam per 5 mL. Meloxicam is chemically designated as 4-hydroxy-2-methyl-*N*-(5-methyl-2-thiazolyl)-2*H*-1,2-benzothiazine-3-carboxamide-1,1-

dioxide. The molecular weight is 351.4. Its empirical formula is $C_{14}H_{13}N_3O_4S_2$ and it has the following structural formula.



Meloxicam is a pastel yellow solid, practically insoluble in water, with higher solubility observed in strong acids and bases. It is very slightly soluble in methanol. Meloxicam has an apparent partition coefficient $(\log P)_{app} = 0.1$ in *n*-octanol/buffer pH 7.4. Meloxicam has pKa values of 1.1 and 4.2.

MOBIC is available as a tablet for oral administration containing 7.5 mg or 15 mg meloxicam, and as an oral suspension containing 7.5 mg meloxicam per 5 mL.

The inactive ingredients in Mobic® (meloxicam) tablets include colloidal silicon dioxide, crospovidone, lactose monohydrate, magnesium stearate, microcrystalline cellulose, povidone and sodium citrate dihydrate.

The inactive ingredients in Mobic-® (meloxicam) oral suspension include colloidal silicon dioxide, hydroxyethylcellulose, sorbitol, glycerol, xylitol, monobasic sodium phosphate (dihydrate), saccharin sodium, sodium benzoate, citric acid (monohydrate), raspberry flavor, and purified water.

CLINICAL PHARMACOLOGY

Mechanism of Action

Meloxicam is a nonsteroidal anti-inflammatory drug (NSAID) that exhibits anti-inflammatory, analgesic, and antipyretic activities in animal models. The mechanism of action of meloxicam, like that of other NSAIDs, may be related to prostaglandin synthetase (cyclo-oxygenase) inhibition.

Pharmacokinetics

Absorption

The absolute bioavailability of meloxicam capsules was 89% following a single oral dose of 30 mg compared with 30 mg IV bolus injection. Following single intravenous doses, dose-proportional pharmacokinetics were shown in the range of 5 mg to 60 mg. After multiple oral doses the pharmacokinetics of meloxicam capsules were dose-proportional over the range of 7.5 mg to 15 mg. Mean C_{max} was achieved within four to five hours after a 7.5 mg meloxicam tablet was taken under fasted conditions, indicating a prolonged drug absorption. With multiple dosing, steady state concentrations were reached by Day 5. A second meloxicam concentration peak occurs around 12 to 14 hours post-dose suggesting biliary recycling.

Meloxicam oral suspension doses of 7.5 mg/5 mL and 15 mg/10 mL have been found to be bioequivalent to meloxicam 7.5 mg and 15 mg capsules, respectively. Meloxicam capsules have been shown to be bioequivalent to Mobic® (meloxicam) tablets.

Table 1 Single Dose and Steady State Pharmacokinetic Parameters for Oral 7.5 mg and 15 mg Meloxicam (Mean and % CV)[1]

		Steady State			Single Dose	
Pharmacokinetic Parameters (% CV)		**Healthy male adults (Fed)[2]**	**Elderly males (Fed)[2]**	**Elderly females (Fed)[2]**	**Renal failure (Fasted)**	**Hepatic insufficiency (Fasted)**
		7.5 mg[3] tablets	**15 mg capsules**	**15 mg capsules**	**15 mg capsules**	**15 mg capsules**
N		**18**	**5**	**8**	**12**	**12**
C_{max}	[μg/mL]	1.05 (20)	2.3 (59)	3.2 (24)	0.59 (36)	0.84 (29)
t_{max}	[h]	4.9 (8)	5 (12)	6 (27)	4 (65)	10 (87)
$t_{1/2}$	[h]	20.1 (29)	21 (34)	24 (34)	18 (46)	16 (29)
CL/f	[mL/min]	8.8 (29)	9.9 (76)	5.1 (22)	19 (43)	11 (44)
V_Z/f^4	[L]	14.7 (32)	15 (42)	10 (30)	26 (44)	14 (29)

[1]The parameter values in the Table are from various studies
[2]not under high fat conditions
[3]MOBIC tablets
[4] $V_Z/f = \text{Dose}/(\text{AUC}\cdot K_{el})$

Food and Antacid Effects

Administration of meloxicam capsules following a high fat breakfast (75 g of fat) resulted in mean peak drug levels (i.e., C_{max}) being increased by approximately 22% while the extent of absorption (AUC) was unchanged. The time to maximum concentration (T_{max}) was achieved between 5 and 6 hours. In comparison, neither the AUC nor the C_{max} values for meloxicam suspension were affected following a similar high fat meal, while mean T_{max} values were increased to approximately 7 hours. No pharmacokinetic interaction was detected with concomitant administration of antacids. Based on these results, MOBIC tablets/oral suspension can be administered without regard to timing of meals or concomitant administration of antacids.

Distribution

The mean volume of distribution (Vss) of meloxicam is approximately 10 L. Meloxicam is ~ 99.4% bound to human plasma proteins (primarily albumin) within the therapeutic dose range. The fraction of protein binding is independent of drug concentration, over the clinically relevant concentration range, but decreases to ~ 99% in patients with renal disease. Meloxicam penetration into human red blood cells, after oral dosing, is less than 10%. Following a radiolabeled dose, over 90% of the radioactivity detected in the plasma was present as unchanged meloxicam.

Meloxicam concentrations in synovial fluid, after a single oral dose, range from 40% to 50% of those in plasma. The free fraction in synovial fluid is 2.5 times higher than in plasma, due to the lower albumin content in synovial fluid as compared to plasma. The significance of this penetration is unknown.

Metabolism
Meloxicam is almost completely metabolized to four pharmacologically inactive metabolites. The major metabolite, 5'-carboxy meloxicam (60% of dose), from P-450 mediated metabolism was formed by oxidation of an intermediate metabolite 5'-hydroxymethyl meloxicam which is also excreted to a lesser extent (9% of dose). *In vitro* studies indicate that cytochrome P-450 2C9 plays an important role in this metabolic pathway with a minor contribution of the CYP 3A4 isozyme. Patients' peroxidase activity is probably responsible for the other two metabolites which account for 16% and 4% of the administered dose, respectively.

Excretion
Meloxicam excretion is predominantly in the form of metabolites, and occurs to equal extents in the urine and feces. Only traces of the unchanged parent compound are excreted in the urine (0.2%) and feces (1.6%). The extent of the urinary excretion was confirmed for unlabeled multiple 7.5 mg doses: 0.5%, 6% and 13% of the dose were found in urine in the form of meloxicam, and the 5'-hydroxymethyl and 5'-carboxy metabolites, respectively. There is significant biliary and/or enteral secretion of the drug. This was demonstrated when oral administration of cholestyramine following a single IV dose of meloxicam decreased the AUC of meloxicam by 50%.

The mean elimination half-life ($t_{1/2}$) ranges from 15 hours to 20 hours. The elimination half-life is constant across dose levels indicating linear metabolism within the therapeutic dose range. Plasma clearance ranges from 7 to 9 mL/min.

Special Populations
Pediatric
After single (0.25 mg/kg) dose administration and after achieving steady-state (0.375 mg/kg/day), there was a general trend of approximately 30% lower exposure in younger patients (2-6 years old) as compared to the older patients (7-16 years old). The older patients had meloxicam exposures similar (single dose) or slightly reduced (steady-state) to those in the adult patients, when using AUC values normalized to a dose of 0.25 mg/kg (see **DOSAGE AND ADMINISTRATION**). The meloxicam mean (SD) elimination half-life was 15.2 (10.1) and 13.0 hours (3.0) for the 2-6 year old patients, and 7-16 year old patients, respectively.

In a covariate analysis, utilizing population pharmacokinetics body-weight, but not age, was the single predictive covariate for differences in the meloxicam apparent oral plasma clearance. The body-weight normalized apparent oral clearance values were adequate predictors of meloxicam exposure in pediatric patients.

The pharmacokinetics of Mobic® (meloxicam) tablets/oral suspension in pediatric patients under 2 years of age have not been investigated.

Geriatric
Elderly males (≥ 65 years of age) exhibited meloxicam plasma concentrations and steady state pharmacokinetics similar to young males. Elderly females (≥ 65 years of age) had a 47% higher AUC_{ss} and 32% higher $C_{max,ss}$ as compared to younger females (≤ 55 years of age) after body weight normalization. Despite the increased total concentrations in the elderly females, the adverse event profile was comparable for both elderly patient populations. A smaller free fraction was found in elderly female patients in comparison to elderly male patients.

Gender
Young females exhibited slightly lower plasma concentrations relative to young males. After single doses of 7.5 mg MOBIC, the mean elimination half-life was 19.5 hours for the female group as compared to 23.4 hours for the male group. At steady state, the data were similar (17.9 hours vs. 21.4 hours). This pharmacokinetic difference due to gender is likely to be of little clinical importance. There was linearity of pharmacokinetics and no appreciable difference in the C_{max} or T_{max} across genders.

Hepatic Insufficiency
Following a single 15 mg dose of meloxicam there was no marked difference in plasma concentrations in subjects with mild (Child-Pugh Class I) and moderate (Child-Pugh Class II) hepatic impairment compared to healthy volunteers. Protein binding of meloxicam was not affected by hepatic insufficiency. No dose adjustment is necessary in mild to moderate hepatic insufficiency. Patients with severe hepatic impairment (Child-Pugh Class III) have not been adequately studied.

Renal Insufficiency
Meloxicam pharmacokinetics have been investigated in subjects with different degrees of renal insufficiency. Total drug plasma concentrations decreased with the degree of renal impairment while free AUC values were similar. Total clearance of meloxicam increased in these patients probably due to the increase in free fraction leading to an increased metabolic clearance. There is no need for dose adjustment in patients with mild to moderate renal failure (CrCL >15 mL/min). Patients with severe renal insufficiency have not been adequately studied. The use of MOBIC tablets/oral suspension in subjects with severe renal impairment is not recommended (see **WARNINGS**, **Advanced Renal Disease**).

Hemodialysis
Following a single dose of meloxicam, the free C_{max} plasma concentrations were higher in patients with renal failure on chronic hemodialysis (1% free fraction) in comparison to healthy volunteers (0.3% free fraction). Hemodialysis did not lower the total drug concentration in plasma; therefore, additional doses are not necessary after hemodialysis. Meloxicam is not dialyzable.

CLINICAL TRIALS

Osteoarthritis and Rheumatoid Arthritis

The use of MOBIC for the treatment of the signs and symptoms of osteoarthritis of the knee and hip was evaluated in a 12-week double-blind controlled trial. MOBIC (3.75 mg, 7.5 mg and 15 mg daily) was compared to placebo. The four primary endpoints were investigator's global assessment, patient global assessment, patient pain assessment, and total WOMAC score (a self-administered questionnaire addressing pain, function and stiffness). Patients on MOBIC 7.5 mg daily and MOBIC 15 mg daily showed significant improvement in each of these endpoints compared with placebo.

The use of MOBIC for the management of signs and symptoms of osteoarthritis was evaluated in six double-blind, active-controlled trials outside the U.S. ranging from 4 weeks to 6 months duration. In these trials, the efficacy of MOBIC, in doses of 7.5 mg/day and 15 mg/day, was comparable to piroxicam 20 mg/day and diclofenac SR 100 mg/day and consistent with the efficacy seen in the U.S. trial.

The use of MOBIC for the treatment of the signs and symptoms of rheumatoid arthritis was evaluated in a 12-week double-blind, controlled multinational trial. MOBIC (7.5 mg, 15 mg and 22.5 mg daily) was compared to placebo. The primary endpoint in this study was the ACR20 response rate, a composite measure of clinical, laboratory and functional measures of RA response. Patients receiving MOBIC 7.5 mg and 15 mg daily showed significant improvement in the primary endpoint compared with placebo. No incremental benefit was observed with the 22.5 mg dose compared to the 15 mg dose.

Higher doses of MOBIC (22.5 mg and greater) have been associated with an increased risk of serious GI events; therefore the daily dose of MOBIC should not exceed 15 mg.

Pauciarticular and Polyarticular Course Juvenile Rheumatoid Arthritis (JRA)

The use of MOBIC for the treatment of the signs and symptoms of pauciarticular or polyarticular course Juvenile Rheumatoid Arthritis in patients 2 years of age and older was evaluated in two 12-week, double-blind, parallel-arm, active-controlled trials. Both studies included three arms: naproxen and two doses of meloxicam. In both studies, meloxicam dosing began at 0.125 mg/kg/day (7.5 mg maximum) or 0.25 mg/kg/day (15 mg maximum), and naproxen dosing began at 10 mg/kg/day. One study used these doses throughout the 12-week dosing period, while the other incorporated a titration after 4 weeks to doses of 0.25 mg/kg/day and 0.375 mg/kg/day (22.5 mg maximum) of meloxicam and 15 mg/kg/day of naproxen.

The efficacy analysis used the ACR Pediatric 30 responder definition, a composite of parent and investigator assessments, counts of active joints and joints with limited range of motion, and erythrocyte sedimentation rate. The proportion of responders were similar in all three groups in both studies, and no difference was observed between the meloxicam dose groups.

INDICATIONS AND USAGE

Carefully consider the potential benefits and risks of Mobic® (meloxicam) tablets/oral suspension and other treatment options before deciding to use MOBIC tablets/oral suspension. Use the lowest effective dose for the shortest duration consistent with individual patient treatment goals (see **WARNINGS**).

MOBIC tablets/oral suspension is indicated for relief of the signs and symptoms of osteoarthritis and rheumatoid arthritis.

MOBIC tablets/oral suspension is indicated for relief of the signs and symptoms of pauciarticular or polyarticular course Juvenile Rheumatoid Arthritis in patients 2 years of age and older.

CONTRAINDICATIONS

MOBIC tablets/oral suspension is contraindicated in patients with known hypersensitivity to meloxicam.

MOBIC tablets/oral suspension should not be given to patients who have experienced asthma, urticaria, or allergic-type reactions after taking aspirin or other NSAIDs. Severe, rarely fatal, anaphylactic-like reactions to NSAIDs have been reported in such patients (see **WARNINGS**, **Anaphylactoid Reactions**, and **PRECAUTIONS**, **Pre-existing Asthma**).

MOBIC tablets/oral suspension is contraindicated for the treatment of peri-operative pain in the setting of coronary artery bypass graft (CABG) surgery (see **WARNINGS**).

WARNINGS

Cardiovascular Effects

Cardiovascular Thrombotic Events

Clinical trials of several COX-2 selective and nonselective NSAIDs of up to three years duration have shown an increased risk of serious cardiovascular (CV) thrombotic events, myocardial infarction, and stroke, which can be fatal. All NSAIDs, both COX-2 selective and nonselective, may have a similar risk. Patients with known CV disease or risk factors for CV disease may be at greater risk. To minimize the potential risk for an adverse CV event in patients treated with an NSAID, the lowest effective dose should be used for the shortest duration possible. Physicians and patients should remain alert for the development of such events, even in the absence of previous CV symptoms. Patients should be informed about the signs and/or symptoms of serious CV events and the steps to take if they occur.

There is no consistent evidence that concurrent use of aspirin mitigates the increased risk of serious CV thrombotic events associated with NSAID use. The concurrent use of

aspirin and an NSAID does increase the risk of serious GI events (see **WARNINGS, Gastrointestinal (GI) Effects - Risk of GI Ulceration, Bleeding, and Perforation**).

Two large, controlled, clinical trials of a COX-2 selective NSAID for the treatment of pain in the first 10-14 days following CABG surgery found an increased incidence of myocardial infarction and stroke (see **CONTRAINDICATIONS**).

Hypertension

NSAIDs, including Mobic® (meloxicam) tablets/oral suspension, can lead to onset of new hypertension or worsening of pre-existing hypertension, either of which may contribute to the increased incidence of CV events. Patients taking thiazides or loop diuretics may have impaired response to these therapies when taking NSAIDs. NSAIDs, including MOBIC tablets/oral suspension, should be used with caution in patients with hypertension. Blood pressure (BP) should be monitored closely during the initiation of NSAID treatment and throughout the course of therapy.

Congestive Heart Failure and Edema

Fluid retention and edema have been observed in some patients taking NSAIDs. MOBIC tablets/oral suspension should be used with caution in patients with fluid retention, hypertension, or heart failure.

Gastrointestinal (GI) Effects - Risk of GI Ulceration, Bleeding, and Perforation

NSAIDs, including MOBIC tablets/oral suspension, can cause serious gastrointestinal (GI) adverse events including inflammation, bleeding, ulceration, and perforation of the stomach, small intestine, or large intestine, which can be fatal. These serious adverse events can occur at any time, with or without warning symptoms, in patients treated with NSAIDs. Only one in five patients, who develop a serious upper GI adverse event on NSAID therapy, is symptomatic. Upper GI ulcers, gross bleeding, or perforation caused by NSAIDs, occur in approximately 1% of patients treated for 3-6 months, and in about 2-4% of patients treated for one year. These trends continue with longer duration of use, increasing the likelihood of developing a serious GI event at some time during the course of therapy. However, even short-term therapy is not without risk.

NSAIDs should be prescribed with extreme caution in those with a prior history of ulcer disease or gastrointestinal bleeding. Patients with a *prior history of peptic ulcer disease and/or gastrointestinal bleeding* who use NSAIDs have a greater than 10-fold increased risk for developing a GI bleed compared to patients with neither of these risk factors. Other factors that increase the risk for GI bleeding in patients treated with NSAIDs include concomitant use of oral corticosteroids or anticoagulants, longer duration of NSAID therapy, smoking, use of alcohol, older age, and poor general health status. Most spontaneous reports of fatal GI events are in elderly or debilitated patients and therefore, special care should be taken in treating this population.

To minimize the potential risk for an adverse GI event in patients treated with an NSAID, the lowest effective dose should be used for the shortest possible duration. Patients and physicians should remain alert for signs and symptoms of GI ulceration and bleeding during NSAID therapy and promptly initiate additional evaluation and treatment if a serious GI adverse event is suspected. This should include discontinuation of the NSAID

until a serious GI adverse event is ruled out. For high-risk patients, alternate therapies that do not involve NSAIDs should be considered.

Renal Effects
Long-term administration of NSAIDs, including Mobic® (meloxicam) tablets/oral suspension, can result in renal papillary necrosis, renal insufficiency, acute renal failure, and other renal injury. Renal toxicity has also been seen in patients in whom renal prostaglandins have a compensatory role in the maintenance of renal perfusion. In these patients, administration of a nonsteroidal anti-inflammatory drug may cause a dose-dependent reduction in prostaglandin formation and, secondarily, in renal blood flow, which may precipitate overt renal decompensation. Patients at greatest risk of this reaction are those with impaired renal function, heart failure, liver dysfunction, those taking diuretics, ACE inhibitors, and angiotensin II receptor antagonists, and the elderly. Discontinuation of NSAID therapy is usually followed by recovery to the pretreatment state.

Advanced Renal Disease
No information is available from controlled clinical studies regarding the use of MOBIC tablets/oral suspension in patients with advanced renal disease. Therefore, treatment with MOBIC tablets/oral suspension is not recommended in these patients with advanced renal disease. If MOBIC tablets/oral suspension therapy must be initiated, close monitoring of the patient's renal function is advisable.

Anaphylactoid Reactions
As with other NSAIDS, anaphylactoid reactions have occurred in patients without known prior exposure to MOBIC tablets/oral suspension. MOBIC tablets/oral suspension should not be given to patients with the aspirin triad. This symptom complex typically occurs in asthmatic patients who experience rhinitis with or without nasal polyps, or who exhibit severe, potentially fatal bronchospasm after taking aspirin or other NSAIDs (see **CONTRAINDICATIONS** and **PRECAUTIONS**, **Pre-existing Asthma**). Emergency help should be sought in cases where an anaphylactoid reaction occurs.

Skin Reactions
NSAIDs, including MOBIC tablets/oral suspension, can cause serious skin adverse events such as exfoliative dermatitis, Stevens-Johnson Syndrome (SJS), and toxic epidermal necrolysis (TEN), which can be fatal. These serious events may occur without warning. Patients should be informed about the signs and symptoms of serious skin manifestations and use of the drug should be discontinued at the first appearance of skin rash or any other sign of hypersensitivity.

Pregnancy
In late pregnancy, as with other NSAIDs, MOBIC tablets/oral suspension should be avoided because it may cause premature closure of the ductus arteriosus.

PRECAUTIONS

General
Mobic® (meloxicam) tablets/oral suspension cannot be expected to substitute for corticosteroids or to treat corticosteroid insufficiency. Abrupt discontinuation of corticosteroids may lead to disease exacerbation. Patients on prolonged corticosteroid therapy should have their therapy tapered slowly if a decision is made to discontinue corticosteroids.

The pharmacological activity of MOBIC tablets/oral suspension in reducing fever and inflammation may diminish the utility of these diagnostic signs in detecting complications of presumed noninfectious, painful conditions.

Hepatic Effects
Borderline elevations of one or more liver tests may occur in up to 15% of patients taking NSAIDs including MOBIC tablets/oral suspension. These laboratory abnormalities may progress, may remain unchanged, or may be transient with continuing therapy. Notable elevations of ALT or AST (approximately three or more times the upper limit of normal) have been reported in approximately 1% of patients in clinical trials with NSAIDs. In addition, rare cases of severe hepatic reactions, including jaundice and fatal fulminant hepatitis, liver necrosis and hepatic failure, some of them with fatal outcomes have been reported.

A patient with symptoms and/or signs suggesting liver dysfunction, or in whom an abnormal liver test has occurred, should be evaluated for evidence of the development of a more severe hepatic reaction while on therapy with MOBIC tablets/oral suspension. If clinical signs and symptoms consistent with liver disease develop, or if systemic manifestations occur (e.g., eosinophilia, rash, etc.), MOBIC tablets/oral suspension should be discontinued.

Renal Effects
Caution should be used when initiating treatment with MOBIC tablets/oral suspension in patients with considerable dehydration. It is advisable to rehydrate patients first and then start therapy with MOBIC tablets/oral suspension. Caution is also recommended in patients with pre-existing kidney disease (see **WARNINGS**, **Renal Effects** and **Advanced Renal Disease**).

The extent to which metabolites may accumulate in patients with renal failure has not been studied with MOBIC tablets/oral suspension. Because some MOBIC tablets/oral suspension metabolites are excreted by the kidney, patients with significantly impaired renal function should be more closely monitored.

Hematological Effects
Anemia is sometimes seen in patients receiving NSAIDs, including MOBIC tablets/oral suspension. This may be due to fluid retention, occult or gross GI blood loss, or an incompletely described effect upon erythropoiesis. Patients on long-term treatment with NSAIDs, including MOBIC tablets/oral suspension, should have their hemoglobin or hematocrit checked if they exhibit any signs or symptoms of anemia.

Drugs which inhibit the biosynthesis of prostaglandins may interfere to some extent with platelet function and vascular responses to bleeding.

NSAIDs inhibit platelet aggregation and have been shown to prolong bleeding time in some patients. Unlike aspirin their effect on platelet function is quantitatively less, of shorter duration, and reversible. Patients receiving Mobic® (meloxicam) tablets/oral suspension who may be adversely affected by alterations in platelet function, such as those with coagulation disorders or patients receiving anticoagulants, should be carefully monitored.

Pre-existing Asthma
Patients with asthma may have aspirin-sensitive asthma. The use of aspirin in patients with aspirin-sensitive asthma has been associated with severe bronchospasm which can be fatal. Since cross reactivity, including bronchospasm, between aspirin and other NSAIDs has been reported in such aspirin-sensitive patients, MOBIC tablets/oral suspension should not be administered to patients with this form of aspirin sensitivity and should be used with caution in patients with pre-existing asthma.

Information for Patients
Patients should be informed of the following information before initiating therapy with an NSAID and periodically during the course of ongoing therapy. Patients should also be encouraged to read the NSAID Medication Guide that accompanies each prescription dispensed.

1. MOBIC tablets/oral suspension, like other NSAIDs, may cause serious CV side effects, such as MI or stroke, which may result in hospitalization and even death. Although serious CV events can occur without warning symptoms, patients should be alert for the signs and symptoms of chest pain, shortness of breath, weakness, slurring of speech, and should ask for medical advice when observing any indicative sign or symptoms. Patients should be apprised of the importance of this follow-up (see **WARNINGS, Cardiovascular Effects**).

2. MOBIC tablets/oral suspension, like other NSAIDs, can cause GI discomfort and, rarely, serious GI side effects, such as ulcers and bleeding, which may result in hospitalization and even death. Although serious GI tract ulcerations and bleeding can occur without warning symptoms, patients should be alert for the signs and symptoms of ulcerations and bleeding, and should ask for medical advice when observing any indicative sign or symptoms including epigastric pain, dyspepsia, melena, and hematemesis. Patients should be apprised of the importance of this follow-up (see **WARNINGS, Gastrointestinal (GI) Effects - Risk of GI Ulceration, Bleeding, and Perforation**).

3. MOBIC tablets/oral suspension, like other NSAIDs, can cause serious skin side effects such as exfoliative dermatitis, SJS, and TEN, which may result in hospitalizations and even death. Although serious skin reactions may occur

without warning, patients should be alert for the signs and symptoms of skin rash and blisters, fever, or other signs of hypersensitivity such as itching, and should ask for medical advice when observing any indicative signs or symptoms. Patients should be advised to stop the drug immediately if they develop any type of rash and contact their physicians as soon as possible.

4. Patients should promptly report signs or symptoms of unexplained weight gain or edema to their physicians.

5. Patients should be informed of the warning signs and symptoms of hepatotoxicity (e.g., nausea, fatigue, lethargy, pruritus, jaundice, right upper quadrant tenderness, and "flu-like" symptoms). If these occur, patients should be instructed to stop therapy and seek immediate medical therapy.

6. Patients should be informed of the signs of an anaphylactoid reaction (e.g., difficulty breathing, swelling of the face or throat). If these occur, patients should be instructed to seek immediate emergency help (see **WARNINGS**).

7. In late pregnancy, as with other NSAIDs, Mobic® (meloxicam) tablets/oral suspension should be avoided because it may cause premature closure of the ductus arteriosus.

Laboratory Tests
Because serious GI tract ulcerations and bleeding can occur without warning symptoms, physicians should monitor for signs or symptoms of GI bleeding. Patients on long-term treatment with NSAIDs should have their CBC and a chemistry profile checked periodically. If clinical signs and symptoms consistent with liver or renal disease develop, systemic manifestations occur (e.g., eosinophilia, rash, etc.) or if abnormal liver tests persist or worsen, MOBIC tablets/oral suspension should be discontinued.

Drug Interactions
ACE-inhibitors
Reports suggest that NSAIDs may diminish the antihypertensive effect of ACE-inhibitors. This interaction should be given consideration in patients taking NSAIDs concomitantly with ACE inhibitors.

Aspirin
When MOBIC tablets/oral suspension is administered with aspirin (1000 mg TID) to healthy volunteers, it tended to increase the AUC (10%) and C_{max} (24%) of meloxicam. The clinical significance of this interaction is not known; however, as with other NSAIDs concomitant administration of meloxicam and aspirin is not generally recommended because of the potential for increased adverse effects.

Concomitant administration of low-dose aspirin with MOBIC tablets/oral suspension may result in an increased rate of GI ulceration or other complications, compared to use

of Mobic® (meloxicam) tablets/oral suspension alone. MOBIC tablets/oral suspension is not a substitute for aspirin for cardiovascular prophylaxis.

Cholestyramine
Pretreatment for four days with cholestyramine significantly increased the clearance of meloxicam by 50%. This resulted in a decrease in $t_{1/2}$, from 19.2 hours to 12.5 hours, and a 35% reduction in AUC. This suggests the existence of a recirculation pathway for meloxicam in the gastrointestinal tract. The clinical relevance of this interaction has not been established.

Cimetidine
Concomitant administration of 200 mg cimetidine QID did not alter the single-dose pharmacokinetics of 30 mg meloxicam.

Digoxin
Meloxicam 15 mg once daily for 7 days did not alter the plasma concentration profile of digoxin after β-acetyldigoxin administration for 7 days at clinical doses. *In vitro* testing found no protein binding drug interaction between digoxin and meloxicam.

Furosemide
Clinical studies, as well as post-marketing observations, have shown that NSAIDs can reduce the natriuretic effect of furosemide and thiazides in some patients. This response has been attributed to inhibition of renal prostaglandin synthesis. Studies with furosemide agents and meloxicam have not demonstrated a reduction in natriuretic effect. Furosemide single and multiple dose pharmacodynamics and pharmacokinetics are not affected by multiple doses of meloxicam. Nevertheless, during concomitant therapy with MOBIC tablets/oral suspension, patients should be observed closely for signs of renal failure (see **WARNINGS**, **Renal Effects**), as well as to assure diuretic efficacy.

Lithium
In a study conducted in healthy subjects, mean pre-dose lithium concentration and AUC were increased by 21% in subjects receiving lithium doses ranging from 804 to 1072 mg BID with meloxicam 15 mg QD as compared to subjects receiving lithium alone. These effects have been attributed to inhibition of renal prostaglandin synthesis by MOBIC tablets/oral suspension. Patients on lithium treatment should be closely monitored for signs of lithium toxicity when MOBIC tablets/oral suspension is introduced, adjusted, or withdrawn.

Methotrexate
NSAIDs have been reported to competitively inhibit methotrexate accumulation in rabbit kidney slices. This may indicate that they could enhance the toxicity of methotrexate. Caution should be used when NSAIDs are administered concomitantly with methotrexate.

A study in 13 rheumatoid arthritis (RA) patients evaluated the effects of multiple doses of meloxicam on the pharmacokinetics of methotrexate taken once weekly. Meloxicam did

not have a significant effect on the pharmacokinetics of single doses of methotrexate. *In vitro*, methotrexate did not displace meloxicam from its human serum binding sites.

Warfarin
The effects of warfarin and NSAIDs on GI bleeding are synergistic, such that users of both drugs together have a risk of serious GI bleeding higher than users of either drug alone.

Anticoagulant activity should be monitored, particularly in the first few days after initiating or changing Mobic® (meloxicam) tablets/oral suspension therapy in patients receiving warfarin or similar agents, since these patients are at an increased risk of bleeding. The effect of meloxicam on the anticoagulant effect of warfarin was studied in a group of healthy subjects receiving daily doses of warfarin that produced an INR (International Normalized Ratio) between 1.2 and 1.8. In these subjects, meloxicam did not alter warfarin pharmacokinetics and the average anticoagulant effect of warfarin as determined by prothrombin time. However, one subject showed an increase in INR from 1.5 to 2.1. Caution should be used when administering MOBIC tablets/oral suspension with warfarin since patients on warfarin may experience changes in INR and an increased risk of bleeding complications when a new medication is introduced.

Carcinogenesis, Mutagenesis, Impairment of Fertility
No carcinogenic effect of meloxicam was observed in rats given oral doses up to 0.8 mg/kg/day (approximately 0.4-fold the human dose at 15 mg/day for a 50 kg adult based on body surface area conversion) for 104 weeks or in mice given oral doses up to 8.0 mg/kg/day (approximately 2.2-fold the human dose, as noted above) for 99 weeks.

Meloxicam was not mutagenic in an Ames assay, or clastogenic in a chromosome aberration assay with human lymphocytes and an *in vivo* micronucleus test in mouse bone marrow.

Meloxicam did not impair male and female fertility in rats at oral doses up to 9 and 5 mg/kg/day, respectively (4.9-fold and 2.5-fold the human dose, as noted above). However, an increased incidence of embryolethality at oral doses ≥ 1 mg/kg/day (0.5-fold the human dose, as noted above) was observed in rats when dams were given meloxicam 2 weeks prior to mating and during early embryonic development.

Pregnancy
Teratogenic Effects: *Pregnancy Category C.*
Meloxicam caused an increased incidence of septal defect of the heart, a rare event, at an oral dose of 60 mg/kg/day (64.5-fold the human dose at 15 mg/day for a 50 kg adult based on body surface area conversion) and embryolethality at oral doses ≥ 5 mg/kg/day (5.4-fold the human dose, as noted above) when rabbits were treated throughout organogenesis. Meloxicam was not teratogenic in rats up to an oral dose of 4 mg/kg/day (approximately 2.2-fold the human dose, as noted above) throughout organogenesis. An increased incidence of stillbirths was observed when rats were given oral doses ≥ 1 mg/kg/day throughout organogenesis. Meloxicam crosses the placental barrier. There

are no adequate and well-controlled studies in pregnant women. Mobic® (meloxicam) tablets/oral suspension should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus.

Nonteratogenic Effects
Because of the known effects of nonsteroidal anti-inflammatory drugs on the fetal cardiovascular system (closure of ductus arteriosus), use during pregnancy (particularly late pregnancy) should be avoided.

Meloxicam caused a reduction in birth index, live births, and neonatal survival at oral doses ≥ 0.125 mg/kg/day (approximately 0.07-fold the human dose at 15 mg/day for a 50 kg adult based on body surface area conversion) when rats were treated during the late gestation and lactation period. No studies have been conducted to evaluate the effect of meloxicam on the closure of the ductus arteriosus in humans; use of meloxicam during the third trimester of pregnancy should be avoided.

Labor and Delivery
Studies in rats with meloxicam, as with other drugs known to inhibit prostaglandin synthesis, showed an increased incidence of stillbirths, prolonged delivery, and delayed parturition at oral dosages ≥ 1 mg/kg/day (approximately 0.5-fold the human dose at 15 mg/day for a 50 kg adult based on body surface area conversion), and decreased pup survival at an oral dose of 4 mg/kg/day (approximately 2.1-fold the human dose, as noted above) throughout organogenesis. Similar findings were observed in rats receiving oral dosages ≥ 0.125 mg/kg/day (approximately 0.07-fold the human dose, as noted above) during late gestation and the lactation period.

The effects of MOBIC tablets/oral suspension on labor and delivery in pregnant women are unknown.

Nursing Mothers
It is not known whether this drug is excreted in human milk however, meloxicam was excreted in the milk of lactating rats at concentrations higher than those in plasma. Because many drugs are excreted in human milk and because of the potential for serious adverse reactions in nursing infants from MOBIC tablets/oral suspension, a decision should be made whether to discontinue nursing or to discontinue the drug, taking into account the importance of the drug to the mother.

Pediatric Use
The safety and effectiveness of meloxicam in pediatric JRA patients from 2 to 17 years of age has been evaluated in three clinical trials (see **CLINICAL TRIALS, ADVERSE REACTIONS** and **DOSAGE AND ADMINISTRATION** sections).

Geriatric Use
As with any NSAID, caution should be exercised in treating the elderly (65 years and older).

ADVERSE REACTIONS

Adults

Osteoarthritis and Rheumatoid Arthritis

The MOBIC Phase 2/3 clinical trial database includes 10,122 OA patients and 1012 RA patients treated with MOBIC 7.5 mg/day, 3,505 OA patients and 1351 RA patients treated with MOBIC 15 mg/day. MOBIC at these doses was administered to 661 patients for at least 6 months and to 312 patients for at least one year. Approximately 10,500 of these patients were treated in ten placebo and/or active-controlled osteoarthritis trials and 2363 of these patients were treated in ten placebo and/or active-controlled rheumatoid arthritis trials. Gastrointestinal (GI) adverse events were the most frequently reported adverse events in all treatment groups across MOBIC trials.

A 12-week multicenter, double-blind, randomized trial was conducted in patients with osteoarthritis of the knee or hip to compare the efficacy and safety of MOBIC with placebo and with an active control. Two 12-week multicenter, double-blind, randomized trials were conducted in patients with rheumatoid arthritis to compare the efficacy and safety of MOBIC with placebo.

Table 2a depicts adverse events that occurred in ≥ 2% of the MOBIC treatment groups in a 12-week placebo and active-controlled osteoarthritis trial.

Table 2b depicts adverse events that occurred in ≥ 2% of the MOBIC treatment groups in two 12-week placebo controlled rheumatoid arthritis trials.

Table 2a Adverse Events (%) Occurring in ≥ 2% of MOBIC Patients in a 12-Week Osteoarthritis Placebo and Active-Controlled Trial

	Placebo	**MOBIC 7.5 mg daily**	**MOBIC 15 mg daily**	**Diclofenac 100 mg daily**
No. of Patients	**157**	**154**	**156**	**153**
Gastrointestinal	17.2	20.1	17.3	28.1
Abdominal Pain	2.5	1.9	2.6	1.3
Diarrhea	3.8	7.8	3.2	9.2
Dyspepsia	4.5	4.5	4.5	6.5
Flatulence	4.5	3.2	3.2	3.9
Nausea	3.2	3.9	3.8	7.2
Body as a Whole				
Accident Household	1.9	4.5	3.2	2.6
Edema[1]	2.5	1.9	4.5	3.3
Fall	0.6	2.6	0.0	1.3
Influenza-Like Symptoms	5.1	4.5	5.8	2.6
Central and Peripheral Nervous System				
Dizziness	3.2	2.6	3.8	2.0
Headache	10.2	7.8	8.3	5.9
Respiratory				
Pharyngitis	1.3	0.6	3.2	1.3

Upper Respiratory Tract Infection	1.9	3.2	1.9	3.3
Skin				
Rash[2]	2.5	2.6	0.6	2.0

[1] WHO preferred terms edema, edema dependent, edema peripheral and edema legs combined
[2] WHO preferred terms rash, rash erythematous and rash maculo-papular combined

Table 2b Adverse Events (%) Occurring in ≥ 2% of MOBIC Patients in two 12-Week Rheumatoid Arthritis Placebo Controlled Trials

	Placebo	MOBIC 7.5 mg daily	MOBIC 15 mg daily
No. of Patients	**469**	**481**	**477**
Gastrointestinal disorders	14.1	18.9	16.8
Abdominal pain NOS[2]	0.6	2.9	2.3
Diarrhea NOS[2]	5.1	4.8	3.4
Dyspeptic signs and symptoms[1]	3.8	5.8	4.0
Nausea[2]	2.6	3.3	3.8
General disorders and administration site conditions			
Influenza like illness[2]	2.1	2.9	2.3
Infection and infestations			
Upper respiratory tract infections-pathogen class unspecified[1]	4.1	7.0	6.5
Musculoskeletal and connective tissue disorders			
Joint related signs and symptoms [1]	1.9	1.5	2.3
Musculoskeletal and connective tissue signs and symptoms NEC[1]	3.8	1.7	2.9
Nervous system disorders			
Headaches NOS[2]	6.4	6.4	5.5
Dizziness (excl vertigo) [2]	3.0	2.3	0.4
Skin and subcutaneous tissue disorders			
Rash NOS[2]	1.7	1.0	2.1

[1] MedDRA high level term (preferred terms): dyspeptic signs and symptoms (dyspepsia, dyspepsia aggravated, eructation, gastrointestinal irritation), upper respiratory tract infections-pathogen unspecified (laryngitis NOS, pharyngitis NOS, sinusitis NOS), joint related signs and symptoms (arthralgia, arthralgia aggravated, joint crepitation, joint effusion, joint swelling), and musculoskeletal and connective tissue signs and symptoms NEC (back pain, back pain aggravated, muscle spasms, musculoskeletal pain)
[2] MedDRA preferred term: diarrhea NOS, nausea, abdominal pain NOS, influenza like illness, headaches NOS, dizziness (excl vertigo), and rash NOS

The adverse events that occurred with MOBIC in ≥ 2% of patients treated short-term (4-6 weeks) and long-term (6 months) in active-controlled osteoarthritis trials are presented in Table 3.

Table 3 Adverse Events (%) Occurring in ≥ 2% of MOBIC Patients in 4 to 6 Weeks and 6 Month Active-Controlled Osteoarthritis Trials

	4-6 Weeks Controlled Trials		6 Month Controlled Trials	
	MOBIC 7.5 mg daily	MOBIC 15 mg daily	MOBIC 7.5 mg daily	MOBIC 15 mg daily
No. of Patients	**8955**	**256**	**169**	**306**
Gastrointestinal	11.8	18.0	26.6	24.2
Abdominal Pain	2.7	2.3	4.7	2.9
Constipation	0.8	1.2	1.8	2.6
Diarrhea	1.9	2.7	5.9	2.6
Dyspepsia	3.8	7.4	8.9	9.5
Flatulence	0.5	0.4	3.0	2.6
Nausea	2.4	4.7	4.7	7.2
Vomiting	0.6	0.8	1.8	2.6
Body as a Whole				
Accident Household	0.0	0.0	0.6	2.9
Edema[1]	0.6	2.0	2.4	1.6
Pain	0.9	2.0	3.6	5.2
Central and Peripheral Nervous System				
Dizziness	1.1	1.6	2.4	2.6
Headache	2.4	2.7	3.6	2.6
Hematologic				
Anemia	0.1	0.0	4.1	2.9
Musculoskeletal				
Arthralgia	0.5	0.0	5.3	1.3
Back Pain	0.5	0.4	3.0	0.7
Psychiatric				
Insomnia	0.4	0.0	3.6	1.6
Respiratory				
Coughing	0.2	0.8	2.4	1.0
Upper Respiratory Tract Infection	0.2	0.0	8.3	7.5
Skin				
Pruritus	0.4	1.2	2.4	0.0
Rash[2]	0.3	1.2	3.0	1.3
Urinary				
Micturition Frequency	0.1	0.4	2.4	1.3
Urinary Tract Infection	0.3	0.4	4.7	6.9

[1] WHO preferred terms edema, edema dependent, edema peripheral and edema legs combined

[2] WHO preferred terms rash, rash erythematous and rash maculo-papular combined

Higher doses of MOBIC (22.5 mg and greater) have been associated with an increased risk of serious GI events; therefore the daily dose of MOBIC should not exceed 15 mg.

Pediatrics

Pauciarticular and Polyarticular Course Juvenile Rheumatoid Arthritis (JRA)

Three hundred and eighty-seven patients with pauciarticular and polyarticular course JRA were exposed to MOBIC with doses ranging from 0.125 to 0.375 mg/kg per day in three clinical trials. These studies consisted of two 12-week multicenter, double-blind, randomized trials (one with a 12-week open-label extension and one with a 40-week extension) and one 1-year open-label PK study. The adverse events observed in these pediatric studies with MOBIC were similar in nature to the adult clinical trial experience, although there were differences in frequency. In particular, the following most common adverse events, abdominal pain, vomiting, diarrhea, headache, and pyrexia, were more common in the pediatric than in the adult trials. Rash was reported in seven (<2%) patients receiving MOBIC. No unexpected adverse events were identified during the course of the trials. The adverse events did not demonstrate an age or gender-specific subgroup effect.

The following is a list of adverse drug reactions occurring in < 2% of patients receiving MOBIC in clinical trials involving approximately 16,200 patients. Adverse reactions reported only in worldwide post-marketing experience or the literature are shown in italics~~ and are considered rare (< 0.1%)~~.

Body as a Whole	allergic reaction, *anaphylactoid reactions including shock,* face edema, fatigue, fever, hot flushes, malaise, syncope, weight decrease, weight increase
Cardiovascular	angina pectoris, cardiac failure, hypertension, hypotension, myocardial infarction, vasculitis
Central and Peripheral Nervous System	convulsions, paresthesia, tremor, vertigo
Gastrointestinal	colitis, dry mouth, duodenal ulcer, eructation, esophagitis, gastric ulcer, gastritis, gastroesophageal reflux, gastrointestinal hemorrhage, hematemesis, hemorrhagic duodenal ulcer, hemorrhagic gastric ulcer, intestinal perforation, melena, pancreatitis, perforated duodenal ulcer, perforated gastric ulcer, stomatitis ulcerative
Heart Rate and Rhythm	arrhythmia, palpitation, tachycardia
Hematologic	*agranulocytosis,* leukopenia, purpura, thrombocytopenia
Liver and Biliary System	ALT increased, AST increased, bilirubinemia, GGT increased, hepatitis, *jaundice, liver failure*
Metabolic and Nutritional	dehydration

Psychiatric ~~Disorders~~	abnormal dreaming, *alterations in mood (such as mood elevation),* anxiety, appetite increased, confusion, depression, nervousness, somnolence
Respiratory	asthma, bronchospasm, dyspnea
Skin and Appendages	alopecia, angioedema, bullous eruption, *erythema multiforme,* photosensitivity reaction, pruritus, *exfoliative dermatitis, Stevens-Johnson syndrome,* sweating increased, *toxic epidermal necrolysis,* urticaria
Special Senses	abnormal vision, conjunctivitis, taste perversion, tinnitus
Urinary System	*acute urinary retention*, albuminuria, BUN increased, creatinine increased, hematuria, *interstitial nephritis,* renal failure

OVERDOSAGE

There is limited experience with meloxicam overdose. Four cases have taken 6 to 11 times the highest recommended dose; all recovered. Cholestyramine is known to accelerate the clearance of meloxicam.

Symptoms following acute NSAID overdose are usually limited to lethargy, drowsiness, nausea, vomiting, and epigastric pain, which are generally reversible with supportive care. Gastrointestinal bleeding can occur. Severe poisoning may result in hypertension, acute renal failure, hepatic dysfunction, respiratory depression, coma, convulsions, cardiovascular collapse, and cardiac arrest. Anaphylactoid reactions have been reported with therapeutic ingestion of NSAIDs, and may occur following an overdose.

Patients should be managed with symptomatic and supportive care following an NSAID overdose. In cases of acute overdose, gastric lavage followed by activated charcoal is recommended. Gastric lavage performed more than one hour after overdose has little benefit in the treatment of overdose. Administration of activated charcoal is recommended for patients who present 1-2 hours after overdose. For substantial overdose or severely symptomatic patients, activated charcoal may be administered repeatedly. Accelerated removal of meloxicam by 4 gm oral doses of cholestyramine given three times a day was demonstrated in a clinical trial. Administration of cholestyramine may be useful following an overdose. Forced diuresis, alkalinization of urine, hemodialysis, or hemoperfusion may not be useful due to high protein binding.

DOSAGE AND ADMINISTRATION

Osteoarthritis and Rheumatoid Arthritis

Carefully consider the potential benefits and risks of Mobic® (meloxicam) tablets/oral suspension and other treatment options before deciding to use MOBIC tablets/oral suspension. Use the lowest effective dose for the shortest duration consistent with individual patient treatment goals (see **WARNINGS**).

After observing the response to initial therapy with MOBIC tablets/oral suspension, the dose should be adjusted to suit an individual patient's needs.

For the relief of the signs and symptoms of osteoarthritis the recommended starting and maintenance oral dose of MOBIC is 7.5 mg once daily. Some patients may receive additional benefit by increasing the dose to 15 mg once daily. For the relief of the signs and symptoms of rheumatoid arthritis, the recommended starting and maintenance oral dose of MOBIC is 7.5 mg once daily. Some patients may receive additional benefit by increasing the dose to 15 mg once daily.

MOBIC oral suspension 7.5 mg/5 mL or 15 mg/10 mL may be substituted for MOBIC tablets 7.5 mg or 15 mg, respectively.

The maximum recommended daily oral dose of MOBIC is 15 mg regardless of formulation.

Pauciarticular and Polyarticular Course Juvenile Rheumatoid Arthritis (JRA)

MOBIC oral suspension is available in the strength of 7.5 mg/5 mL. To improve dosing accuracy in smaller weight children, the use of the MOBIC oral suspension is recommended. For the treatment of juvenile rheumatoid arthritis, the recommended oral dose of MOBIC is 0.125 mg/kg once daily up to a maximum of 7.5 mg. There was no additional benefit demonstrated by increasing the dose above 0.125 mg/kg once daily in these clinical trials.

Juvenile Rheumatoid Arthritis dosing using the oral suspension should be individualized based on the weight of the child:

	0.125 mg/kg	
Weight	**Dose (1.5 mg/mL)**	**Delivered dose**
12 kg (26 lb)	1.0 mL	1.5 mg
24 kg (54 lb)	2.0 mL	3.0 mg
36 kg (80 lb)	3.0 mL	4.5 mg
48 kg (106 lb)	4.0 mL	6.0 mg
≥60 kg (132 lb)	5.0 mL	7.5 mg

Shake the oral suspension gently before using.

Mobic® (meloxicam) tablets/oral suspension may be taken without regard to timing of meals.

HOW SUPPLIED

MOBIC is available as a pastel yellow, round, biconvex, uncoated tablet containing meloxicam 7.5 mg or as a pastel yellow, oblong, biconvex, uncoated tablet containing meloxicam 15 mg. The 7.5 mg tablet is impressed with the Boehringer Ingelheim logo on one side, and on the other side, the letter "M". The 15 mg tablet is impressed with the tablet code "15" on one side and the letter "M" on the other. MOBIC is also available as a yellowish green tinged viscous oral suspension containing 7.5 mg meloxicam in 5 mL.

MOBIC tablets 7.5 mg is available as follows:
NDC 0597-0029-01; Bottles of 100

MOBIC tablets 15 mg is available as follows:
NDC 0597-0030-01; Bottles of 100

MOBIC oral suspension 7.5_mg/5_mL is available as follows:
NDC 0597-0034-01; Bottles of 100 mL

Store at 25°C (77°F); excursions permitted to 15°C-30°C (59°F-86°F). Keep MOBIC tablets in a dry place.

Please address medical inquiries to http://us.boehringer-ingelheim.com, (800) 542-6257 or (800) 459-9906 TTY.

Dispense tablets in a tight container. Keep oral suspension container tightly closed.

Keep this and all medications out of the reach of children.

Mobic tablets 7.5 mg and 15 mg are manufactured by:

Boehringer Ingelheim Pharma GmbH & Co. KG
Ingelheim, Germany
and
Boehringer Ingelheim Promeco
S.A. de C.V., Mexico City, Mexico

Mobic oral suspension 7.5 mg/5 mL is manufactured by:

	Boehringer Ingelheim Roxane, Inc. Columbus, OH 43216 USA
Marketed by:	Boehringer Ingelheim Pharmaceuticals, Inc. Ridgefield, CT 06877 USA
Licensed from:	Boehringer Ingelheim International GmbH

U.S. Patent No. 6,184,220 covers the Meloxicam Oral Suspension product.

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Medication Guide for Non-Steroidal Anti-Inflammatory Drugs (NSAIDs.)

(See the end of this Medication Guide for a list of prescription NSAID medicines.)

What is the most important information I should know about medicines called Non-Steroidal Anti-Inflammatory Drugs (NSAIDs)?

NSAID medicines may increase the chance of a heart attack or stroke that can lead to death. This chance increases:

- with longer use of NSAID medicines
- in people who have heart disease

NSAID medicines should never be used right before or after a heart surgery called a "coronary artery bypass graft (CABG)."

NSAID medicines can cause ulcers and bleeding in the stomach and intestines at any time during treatment. Ulcers and bleeding:

- can happen without warning symptoms
- may cause death

The chance of a person getting an ulcer or bleeding increases with:

- taking medicines called "corticosteroids" and "anticoagulants"
- longer use
- smoking
- drinking alcohol
- older age
- having poor health

NSAID medicines should only be used:

- exactly as prescribed
- at the lowest dose possible for your treatment
- for the shortest time needed

What are Non-Steroidal Anti-Inflammatory Drugs (NSAIDs)?

NSAID medicines are used to treat pain and redness, swelling, and heat (inflammation) from medical conditions such as:

- different types of arthritis
- menstrual cramps and other types of short-term pain

Who should not take a Non-Steroidal Anti-Inflammatory Drug (NSAID)?

Do not take an NSAID medicine:

- if you had an asthma attack, hives, or other allergic reaction with aspirin or any other NSAID medicine
- for pain right before or after heart bypass surgery

Tell your healthcare provider:

- about all of your medical conditions.
- about all of the medicines you take. NSAIDs and some other medicines can interact with each other and cause serious side effects. **Keep a list of your medicines to show to your healthcare provider and pharmacist.**
- if you are pregnant. **NSAID medicines should not be used by pregnant women late in their pregnancy.**
- if you are breastfeeding. **Talk to your doctor.**

What are the possible side effects of Non-Steroidal Anti-Inflammatory Drugs (NSAIDs)?

Serious side effects include:

- heart attack
- stroke
- high blood pressure
- heart failure from body swelling (fluid retention)
- kidney problems including kidney failure
- bleeding and ulcers in the stomach and intestine
- low red blood cells (anemia)
- life-threatening skin reactions
- life-threatening allergic reactions
- liver problems including liver failure
- asthma attacks in people who have asthma

Other side effects include:

- stomach pain
- constipation
- diarrhea
- gas
- heartburn
- nausea
- vomiting
- dizziness

Get emergency help right away if you have any of the following symptoms:

- shortness of breath or trouble breathing
- chest pain
- weakness in one part or side of your body
- slurred speech
- swelling of the face or throat

Stop your NSAID medicine and call your healthcare provider right away if you have any of the following symptoms:

- nausea
- more tired or weaker than usual
- itching
- your skin or eyes look yellow
- stomach pain
- flu-like symptoms
- vomit blood
- there is blood in your bowel movement or it is black and sticky like tar
- skin rash or blisters with fever
- unusual weight gain
- swelling of the arms and legs, hands and feet

These are not all the side effects with NSAID medicines. Talk to your healthcare provider or pharmacist for more information about NSAID medicines.

Other information about Non-Steroidal Anti-Inflammatory Drugs (NSAIDs)

Aspirin is an NSAID medicine but it does not increase the chance of a heart attack. Aspirin can cause bleeding in the brain, stomach, and intestines. Aspirin can also cause ulcers in the stomach and intestines. Some of these NSAID medicines are sold in lower doses without a prescription (over-the-counter). Talk to your healthcare provider before using over-the-counter NSAIDs for more than 10 days.

NSAID medicines that need a prescription

Generic Name	Tradename
Celecoxib	Celebrex
Diclofenac	Cataflam, Voltaren, Arthrotec (combined with misoprostol)
Diflunisal	Dolobid
Etodolac	Lodine, Lodine XL
Fenoprofen	Nalfon, Nalfon 200
Flurbiprofen	Ansaid
Ibuprofen	Motrin, Tab-Profen, Vicoprofen* (combined with hydrocodone), Combunox (combined with oxycodone)
Indomethacin	Indocin, Indocin SR, Indo-Lemmon, Indomethagan
Ketoprofen	Oruvail
Ketorolac	Toradol
Mefenamic Acid	Ponstel
Meloxicam	Mobic
Nabumetone	Relafen
Naproxen	Naprosyn, Anaprox, Anaprox DS, EC-Naprosyn, Naprelan, PREVACID NapraPAC (copackaged with lansoprazole)
Oxaprozin	Daypro
Piroxicam	Feldene
Sulindac	Clinoril
Tolmetin	Tolectin, Tolectin DS, Tolectin 600

*Vicoprofen contains the same dose of ibuprofen as over-the-counter (OTC) NSAIDs, and is usually used for less than 10 days to treat pain. The OTC NSAID label warns that long term continuous use may increase the risk of heart attack or stroke.

This Medication Guide has been approved by the U.S. Food and Drug Administration.

EXHIBIT 8

PubMed Health. A service of the National Library of Medicine, National Institutes of Health.

AHFS Consumer Medication Information [Internet]. Bethesda (MD): American Society of Health-System Pharmacists; 2000-2011.

Meloxicam (mel ox' i cam)

Last reviewed: September 1, 2010.

Warning
People who take nonsteroidal anti-inflammatory drugs (NSAIDs) (other than aspirin) such as meloxicam may have a higher risk of having a heart attack or a stroke than people who do not take these medications. These events may happen without warning and may cause death. This risk may be higher for people who take NSAIDs for a long time. Tell your doctor if you or anyone in your family has or has ever had heart disease, a heart attack, or a stroke, if you smoke, and if you have

Show full warning

Why is this medication prescribed?

Meloxicam is used to relieve pain, tenderness, swelling, and stiffness caused by osteoarthritis (arthritis caused by a breakdown of the lining of the joints) and rheumatoid arthritis (arthritis caused by swelling of the lining of the joints). Meloxicam is also used to relieve the pain, tenderness,swelling, and stiffness caused by juvenile rheumatoid arthritis (a type of arthritis that affects children) in children 2 years of age and older. Meloxicam is in a class of medications called nonsteroidal anti-inflammatory drugs (NSAIDs). It works by stopping the body's production of a substance that causes pain, fever, and inflammation.

How should this medicine be used?

Meloxicam comes as a tablet and suspension (liquid) to take by mouth. It is usually taken once a day with or without food. Take meloxicam at the same time every day. Follow the directions on your prescription label carefully, and ask your doctor or pharmacist to explain any part you do not understand. Take meloxicam exactly as directed. Do not take more or less of it or take it more often than prescribed by your doctor.

Shake the suspension well before each use to mix the medication evenly.

Other uses for this medicine

Meloxicam is also used sometimes to treat ankylosing spondylitis (arthritis that mainly affects the spine). Talk to your doctor about the possible risks of using this medication for your condition.

This medication may be prescribed for other uses; ask your doctor or pharmacist for more information.

What special precautions should I follow?

Before taking meloxicam,

- tell your doctor and pharmacist if you are allergic to meloxicam, aspirin or other NSAIDs such as ibuprofen (Advil, Motrin) and naproxen (Aleve, Naprosyn), or any other medications.
- tell your doctor and pharmacist what prescription and nonprescription medications, vitamins, nutritional supplements, and herbal products you are taking or plan to take. Be sure to mention the medications listed in the IMPORTANT WARNING section and any of the following: angiotensin-converting enzyme (ACE) inhibitors such as benazepril (Lotensin), captopril (Capoten), enalapril (Vasotec), fosinopril (Monopril), lisinopril (Prinivil, Zestril), and quinapril (Accupril); cholestyramine (Questran); diuretics ('water pills'); lithium (Eskalith, Lithobid, others); and methotrexate (Rheumatrex). Your doctor may need to change the doses of your medications or monitor you carefully for side effects.
- tell your doctor if you have or have ever had asthma, especially if you have frequent stuffed or runny nose or nasal polyps (swelling of the lining of the nose); swelling of the hands, feet, ankles, or lower legs; or kidney or liver disease.
- tell your doctor if you are pregnant, especially if you are in the last few months of your pregnancy, you plan to become pregnant, or you are breast-feeding. If you become pregnant while taking meloxicam, call your doctor.
- if you are having surgery, including dental surgery, tell the doctor or dentist that you are taking meloxicam.

What should I do if I forget a dose?

Take the missed dose as soon as you remember it. However, if it is almost time for the next dose, skip the missed dose and continue your regular dosing schedule. Do not take a double dose to make up for a missed one.

What side effects can this medication cause?

Meloxicam may cause side effects. Tell your doctor if any of these symptoms are severe or do not go away:

diarrhea

constipation

gas

sore throat

cough

runny nose

Some side effects can be serious. If you experience any of the following symptoms, call your doctor immediately. Do not take any more meloxicam until you speak to your doctor:

fever

blisters

rash

hives

itching

swelling of the eyes, face, tongue, lips, throat, arms, hands, feet, ankles, or lower legs

difficulty breathing or swallowing

hoarseness

pale skin

fast heartbeat

unexplained weight gain

nausea

excessive tiredness

lack of energy

- yellowing of the skin or eyes
- pain in the right upper part of the stomach
- flu-like symptoms
- cloudy, discolored, or bloody urine
- back pain
- difficult or painful urination

Meloxicam may cause other side effects. Call your doctor if you have any unusual problems while taking this medication.

If you experience a serious side effect, you or your doctor may send a report to the Food and Drug Administration's (FDA) MedWatch Adverse Event Reporting program online [at http://www.fda.gov/Safety/MedWatch] or by phone [1-800-332-1088].

What storage conditions are needed for this medicine?

Keep this medication in the container it came in, tightly closed, and out of reach of children. Store it at room temperature and away from excess heat and moisture (not in the bathroom). Throw away any medication that is outdated or no longer needed. Talk to your pharmacist about the proper disposal of your medication.

In case of emergency/overdose

In case of overdose, call your local poison control center at 1-800-222-1222. If the victim has collapsed or is not breathing, call local emergency services at 911.

Symptoms of overdose may include:

- lack of energy
- drowsiness
- nausea
- vomiting
- stomach pain
- bloody, black, or tarry stools
- vomit that is bloody or looks like coffee grounds
- difficulty breathing
- seizures
- coma

What other information should I know?

Do not let anyone else take your medication. Ask your pharmacist any questions you have about refilling your prescription.

It is important for you to keep a written list of all of the prescription and nonprescription (over-the-counter) medicines you are taking, as well as any products such as vitamins, minerals, or other dietary supplements. You should bring this list with you each time you visit a doctor or if you are admitted to a hospital. It is also important information to carry with you in case of emergencies.

The following brand names are from RxNorm, a standardized nomenclature for clinical drugs produced by the National Library of Medicine:

Brand names

- Mobic